# The Institute of Paper Chemistry

Appleton, Wisconsin

## Doctor's Dissertation

**Sorption of Xyloglucan Onto Cellulose Fibers**

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# SORPTION OF XYLOGLUCAN ONTO CELLULOSE FIBERS

A thesis submitted by

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# ABSTRACT

Xyloglucan is a hemicellulose found in the primary cell walls of many plant species. Found adjacent to the cellulose, it is believed to function as a cementing material which contributes crosslinks and rigidity to the cellulose framework. The only noncovalent linkage reported in the primary cell wall cellulose-hemicellulose-pectic polysaccharide matrix of the cultured tissues is that between xyloglucan and cellulose. Therefore, the xyloglucan:cellulose association has been of great interest to researchers.

Xyloglucan can be bound to cellulose *in vitro* to simulate this *in vivo* relationship. Previously, the sorption of xyloglucan fragments onto cellulose under non-physiological conditions had been studied, but little information was available on xyloglucan polymer sorption onto cellulose under more natural conditions. This thesis examined the sorption of the xyloglucan polymer isolated from *Tamarindus indica* onto cellulose fibers (cotton linters) in an aqueous environment.

The structure of the xyloglucan isolated from *Tamarindus indica* in this thesis resembled that of other tamarind xyloglucans reported in the literature. Due to the milder isolation procedure employed, the molecular weight of this polymer was much larger than those previously found. No acetyl, pyruvate, methoxyl, or carboxyl groups were found. The molecular weight and certain structural features (e.g., no fucose units) also differed from the xyloglucan found in plant primary cell walls.

When sorbed onto well characterized cotton linters, this xyloglucan exhibited equilibrium sorption within 24 hours. The equilibrium adsorption isotherm was defined. Monolayer sorption occurred. A maximum specific sorption value of 3.9 milligrams of xyloglucan sorbed per gram of cotton linters was calculated using

Langmuir's adsorption isotherm theory. This value was compared with other values found in the literature for similar polymer adsorption studies. The effect of molecular weight on sorption equilibrium was also examined and found to be insignificant over the molecular weight range of the polymer isolated in this thesis.

# INTRODUCTION

## XYLOGLUCANS

The three major chemical components of wood are cellulose, hemicellulose, and lignin. Depending on what tree species is examined, the ratio of these three components will vary. In general, there is 35-60% cellulose, 15-30% hemicellulose, and 15-35% lignin. Hemicellulose is a very broad term that has been used historically to describe various noncellulosic polysaccharides in plants. Different definitions for hemicellulose have confused the matter. Today, researchers have avoided the term, hemicellulose, and are focusing on definitions based on structure or isolation techniques. Table 1 illustrates Aspinall's classification of plant cell wall polysaccharides by their structural families. [1] Note that both xyloglucan and cellulose are members of the glucan family.

Table 1. Aspinall's classification of plant cell wall polysaccharides by structural family.

Glucans

| | |
|---|---|
| Cellulose | β-(1→4) |
| Callose | β-(1→3) |
| Cereal β-D-glucans | β-(1→3)(1→4) |
| Xyloglucan | β-(1→4) and branches |

Rhamnogalacturonans and associated arabinans and arabinogalactans

Mannans, glucomannans and galactoglucomannans

Xylans

Glucuronomannans

Xyloglucans are hemicelluloses commonly found in immature and storage tissues of many plant species. Originally they were termed amyloids because of their starch-like response to iodine. Two different groups of xyloglucans have been identified as seed wall and cell wall xyloglucans. The most readily available group is that isolated from the seeds of various dicotyledons, such as *Tamarindus indica*/ tamarind gum [2,3,4], *Tropaeoleum majus*/nasturtium gum [5], *Annona muricata* gum [6], and *Brassica campestris*/rape seed gum [7].

These xyloglucans act as a reserve food supply for the developing seed and are generally composed of the D-sugars of glucose, xylose, and galactose (see Table 2) linked in almost identical patterns. It has been shown that these polymers are composed of a β-(1→4)-linked glucan backbone that is substituted (C-6) with a limited number of xylosyl residues, either singly, or with a terminal galactosyl residue (C-1 to C-2). Figure 1 illustrates an example of xyloglucan from *Tamarindus indica*.

Table 2. Normalized sugar compositions of some seed xyloglucans.

| Xyloglucan | Normalized Sugar Composition | | |
|---|---|---|---|
| | Glucose | Xylose | Galactose |
| *Tamarindus indica* [2] | 4 | 3 | 1.3 |
| *Tropaeoleum majus* [5] | 4 | 2.7 | 1.3 |
| *Brassica campestris* [7] | 4 | 1.5 | 0.8 |
| *Annona muricata* [6] | 4 | 1 | 1 |

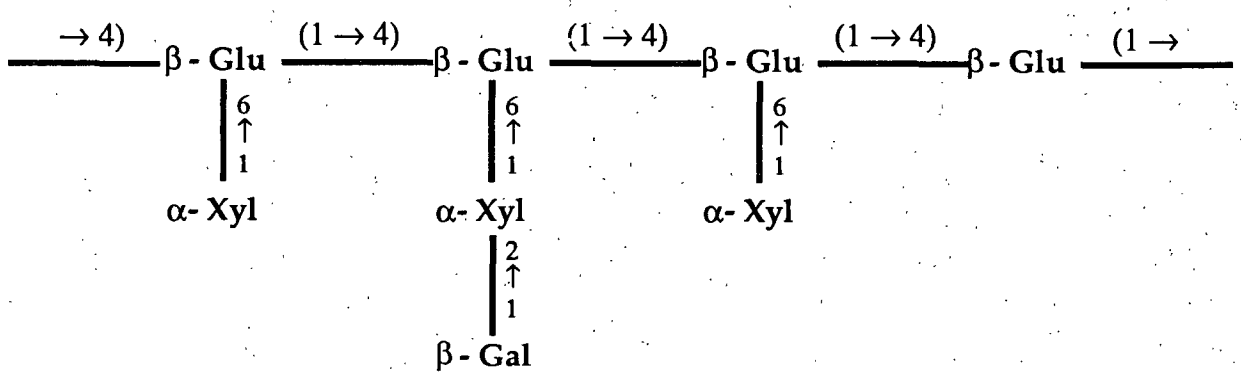


Figure 1. The structure of xyloglucan isolated from *Tamarindus indica*. [2]

The other xyloglucan family has been isolated from the cell walls of various immature monocotyledonous and dicotyledonous tissues. Typical sources include rice hulls [8], apple cell wall material [9], aspen tissue [10], etiolated pea stems [11], and suspension cultured *Rosa glauca* [12], *Acer pseudoplatanus* [13,14], and soybean [15] cells. These xyloglucans often contain terminal fucose units, in addition to glucosyl, xylosyl, and galactosyl sugar moieties (see Table 3). Recently, Nealey [16] isolated a xyloglucan from the spent media of suspension cultured loblolly pine cells, thus demonstrating that this polymer is a component of conifers, as well as angiosperms.

Table 3. Normalized sugar compositions of some cell wall xyloglucans.

| Xyloglucan | Normalized Sugar Composition | | | |
|---|---|---|---|---|
| | Glucose | Xylose | Galactose | Fucose |
| Soybean [15] | 4 | 2.4 | 1.2 | 0.5 |
| Apple [9] | 4 | 2.4 | 1.6 | 0.8 |
| Aspen [10] | 4 | 2.5 | 2.0 | 0.4 |
| *Acer pseudoplatanus* [13] | 4 | 4.0 | - | 1.0 |
| Pea [11] | 4 | 3.0 | 0.5 | 0.5 |
| Loblolly pine [16] | 4 | 1.7 | 1.0 | 0.2 |

The exact nature and function of xyloglucan in the developing plant cell wall have long been of interest to researchers. In 1969, Aspinall *et al.* [13] sparked the curiosity of the scientific community with his concluding remarks on the nature of a fucoxyloglucan from *Acer pseudoplatanus*: "The biological function of such polysaccharides (xyloglucan) is entirely unknown and it is a matter for conjecture whether they are precursors of or transformation products from cellulose." [3] Subsequently, much research has been devoted toward understanding the functions of xyloglucan in the plant cell wall.

## XYLOGLUCAN: FUNCTIONS IN THE PLANT CELL WALL

### Structural Function

Albersheim and coworkers [14,17,18,19] have done considerable research on the primary cell wall of plants. One investigation [14] identified and quantified the macromolecular components of *Acer pseudoplatanus* cell walls (see Table 4). Using enzymatic and chemical degradative techniques, a cell wall xyloglucan from *Acer pseudoplatanus* was isolated and characterized. Their proposed structure for the xyloglucan oligosaccharide they isolated is shown in Figure 2. [17]

Besides isolating and characterizing xyloglucan, Albersheim [14] also reported finding small pieces of xyloglucan bound to galactan chains. These galactans were believed to be the linear side chains of the rhamnogalacturonans (pectic polymers). Albersheim speculated that the galactan, therefore, acts as a bridge between the xyloglucan and the rhamnogalacturonan.

Early work on xyloglucan isolation from suspension culture medium by Aspinall *et al.* [13] indicated that xyloglucan binds strongly, but noncovalently to

Table 4. Polymer composition of suspension cultured *Acer pseudoplatanus* cell walls. [14]

| Wall component | % of cell wall |
|---|---|
| Arabinan | 10 |
| Arabinogalactan | 2 |
| Galactan | 8 |
| Cellulose | 23 |
| Protein | 10 |
| Rhamnogalacturonan | 16 |
| Tetra-arabinosides | 9 |
| Xyloglucan | 21 |
| Total | 99 |

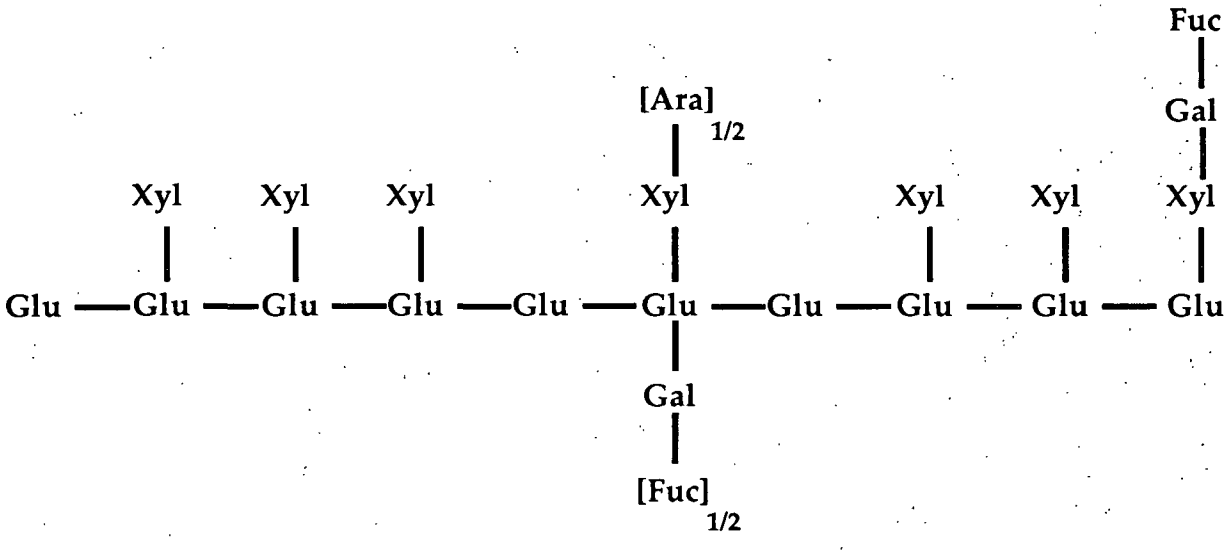


Figure 2. Structure of xyloglucan oligosaccharide from *Acer pseudoplatanus*. [17]

cellulose. This was evident because 7M urea (widely used for the disruption of hydrogen bonds) was needed to release fucoxyloglucan from a cellulose column. [13] Bauer *et al.* [17] found that using urea along with other chemical and enzymatic techniques was necessary for the isolation of xyloglucan from *Acer pseudoplatanus* cell walls. They hypothesized that this noncovalent binding of xyloglucan onto cellulose fibrils in the cell wall could be reversible.

The last two paragraphs illustrate the difficulties Albersheim and his coworkers [17] encountered in isolating and characterizing *Acer pseudoplatanus* cell wall xyloglucan. Due to these difficulties, they hypothesized that xyloglucan is covalently bound to the pectic polysaccharides and noncovalently linked to the cellulose fibrils. From their calculations [17], it was shown that there was enough xyloglucan present in *Acer pseudoplatanus* cell walls to encapsulate the cellulose fibrils in a xyloglucan monolayer. This, they concluded, illustrates an important structural function for xyloglucan in plant cell walls, i.e., that xyloglucan acts as the interconnecting agent between the cellulose fibrils and the pectic polysaccharides.

A tentative model structure for *Acer pseudoplatanus* cell walls was then proposed by Albersheim and coworkers. [19] Figure 3 illustrates the basic features of their model. This model utilizes both xyloglucan-cellulose binding as reported by Aspinall *et al.* [13] and Bauer *et al.* [17], and xyloglucan-galactan covalent bonding. [14] They suggested that a cross-linking of the cell wall structure would also fit their data. [19] Therefore, a single pectic polysaccharide could be attached to more than one cellulose fibril and, conversely, a single cellulose fibril could be attached through xyloglucan chains to more than one pectic polymer. Their plant cell wall model contained this cross-linking feature also.

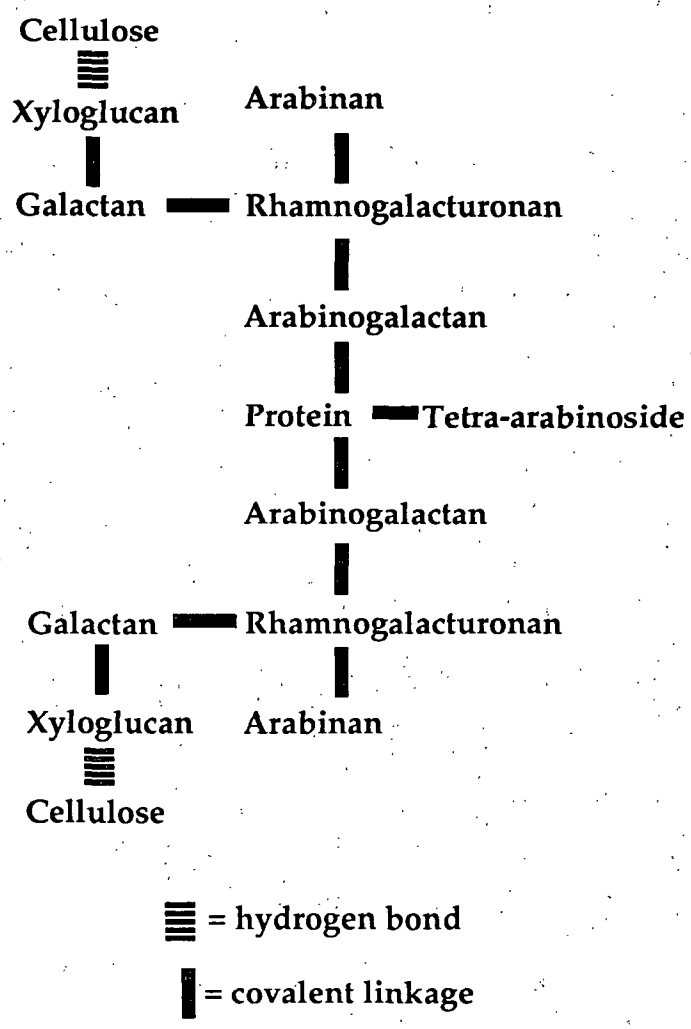


Figure 3. Albersheim's model of the primary cell wall of *Acer pseudoplatanus.* [19]

Other authors have proposed cell wall models [20,21] that differ from Albersheim's. In 1984, two articles supporting separate parts of his cell wall model were published. Hayashi and Maclachlan [11] studied the xyloglucan-cellulose complex obtained from elongating, etiolated pea stems. From their results, support for Albersheim's model can be seen in that "xyloglucan is strongly bound to

cellulose microfibrils by hydrogen bonds" and this xyloglucan-cellulose complex can be regarded as a "naturally occurring multimolecular cross-linked structure in which microfibril surfaces are totally masked and saturated with xyloglucan." [11] Chambat, Barnoud, and Joseleau [12] 1) hypothesized hydrogen bonding between the xyloglucan and cellulose, 2) verified the proposed glycosidic linkage between xyloglucan and the pectic polymers and, 3) presented a model for the cellulose-polysaccharide interconnecting linkages of *Rosa glauca* cell walls (see Figure 4). This model shows a possible xyloglucan to xyloglucan linkage through hydrogen bonding. This disagrees with Albersheim's hypothesis [17] of only a xyloglucan monolayer coating the cellulose fibrils. These two research groups, Hayashi and Maclachlan [11] and Chambat *et al.* [12], presented supporting evidence for different parts of Albersheim's [19] cell wall model.

Thus, the main structural function of xyloglucan in the plant primary cell wall is thought to be the cross-linking of cellulose fibrils and pectic polysaccharides. The ability of the xyloglucan to establish strong intermolecular hydrogen bonds with cellulose is necessary. Therefore, studies concerning the sorption of this hemicellulose onto cellulose fibers would contribute to understanding xyloglucan's role in the plant cell wall.

Proposed Involvement in Cell Wall Elongation

Albersheim *et al.* [19], in their presentation of the cell wall model, hypothesized that the xyloglucan-cellulose relationship was possibly involved in cell wall elongation. Since they believed that the only noncovalent linkage in the cellulose-hemicellulose-pectin complex is between xyloglucan and the cellulose fibril, they could rationalize that cell wall extension resulted from an inch-worm-

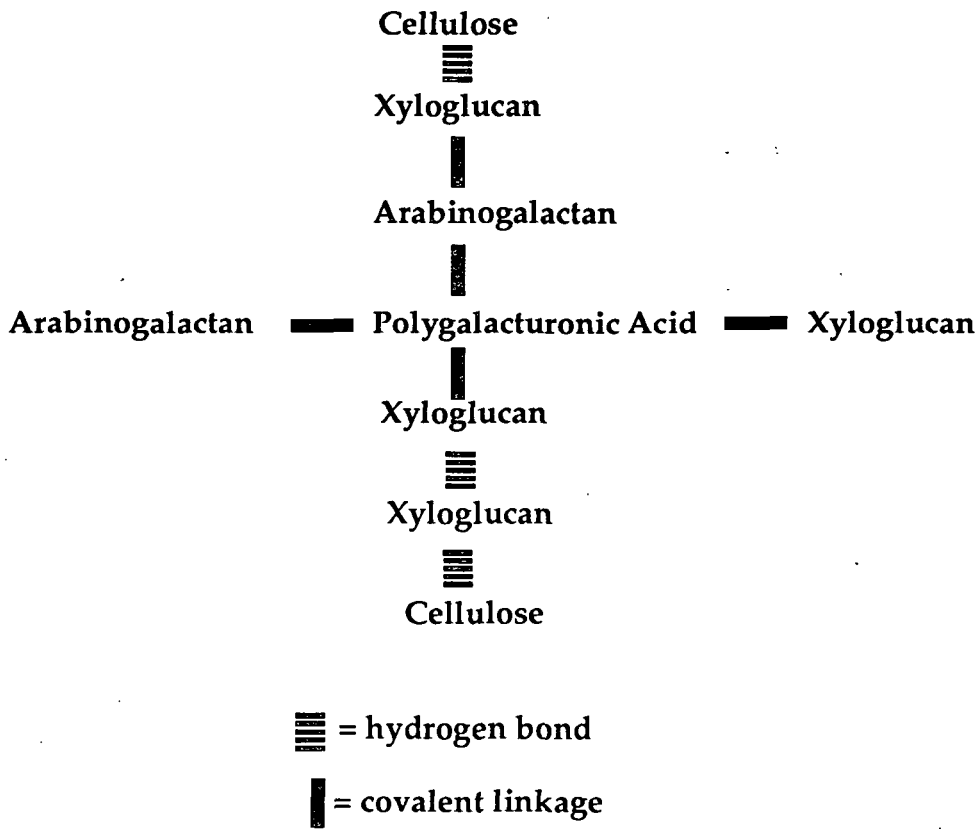


Figure 4. Model of the cellulose-polysaccharide linkages of *Rosa glauca*. [12]

like creep of xyloglucan along the cellulose fibril. This proposed "xyloglucan creep" would require the breaking and reforming of the hydrogen bonds between xyloglucan and the cellulose fibril.

To examine this phenomenon, Valent and Albersheim [18] studied the sorption of seven- and nine-sugar xyloglucan fragments (produced by enzymatic degradation) onto cellulose powder. Intact polymers could not be used in this study, because they bind very strongly and, for experimental purposes, irreversibly to

cellulose in water. These xyloglucan fragments did not bind to cellulose in water, but sorption was promoted by the addition of organic solvents (ethanol and acetone, 50 to 75% organic solvent). Valent and Albersheim [18] believed that this failure of xyloglucan fragments to bind to the cellulose in water illustrated that "portions of the xyloglucan chains within the cell wall can lift off the cellulose fibers, and thereby, initiate creep of the xyloglucan chain."

Valent and Albersheim [18] studied the effect of two variables (temperature and pH) on the sorption of xyloglucan fragments to cellulose powder. These two factors were chosen because of their known effect on cell wall weakening or loosening. Cell wall weakening is the breakdown of the crosslinked cell wall structure and is necessary prior to cell wall elongation. High temperatures weaken intact cell walls. This should correlate with an increase in xyloglucan creep (measured by the decrease in the amount of xyloglucan sorbed or percent xyloglucan bound) if xyloglucan creep is important in cell elongation. They did find approximately a 20% decrease in the amount of sorbed xyloglucan when the temperature was increased from 2°C to 45°C. Lower pH's (i.e., less than pH 5) can cause cell wall weakening and acid-induced elongation. Therefore, if this phenomenon is the rate-limiting step in elongation, increasing the hydrogen ion content should increase xyloglucan creep or decrease the amount sorbed. Table 5 shows that varying the apparent pH between pH 2 to 7 did not affect the binding of xyloglucan fragments to cellulose powder. Apparent pH values could only be obtained due to the presence of the organic solvents used. Thus, Valent and Albersheim's study did illustrate the *in vitro* binding of xyloglucan to cellulose.

Table 5. Effect of apparent pH on the binding of xyloglucan fragments to cellulose in 70% acetone. [18]

| Apparent pH | Bound Xyloglucan Fragments, % |
|---|---|
| 1.9 | 70 |
| 2.6 | 69 |
| 5.0 | 68 |
| 6.7 | 68 |

In another study, Hayashi and coworkers [22] examined aqueous xyloglucan-cellulose interactions *in vitro* by using radioiodinated pea xyloglucan. This xyloglucan had been isolated and characterized (e.g., 330,000 molecular weight) in a previous study. [11] From their sorption experiments, they found monolayer adsorption and approximated the maximum equilibrium amount at 5 micrograms of pea xyloglucan sorbed per 100 micrograms of pea cellulose. Above pH 6, they demonstrated that sorption of xyloglucan onto cellulose was inhibited. The binding of xyloglucan to cellulose was found to be very specific and not affected by the presence of other β-glucans.

Hayashi *et al.* [22] observed *in vivo* the effect of 0.1% pea xyloglucan addition on *Acetobacter xylinum*, a cellulose synthesizing bacterium. The cellulose ribbon structure was partially disrupted in much the same manner as Haigler and Benziman [23] saw when carboxymethylcellulose was added. Both of these polymers associate with the cellulose ribbon subunits and interfere with the formation of larger bundles. Xyloglucan is only found in the primary cell wall where Hayashi [22] reported that pea stem microfibril diameters are very small. This would suggest that

xyloglucan, may in fact, associate with the cellulose to prevent larger bundle formation from taking place.

Other researchers have noted that xyloglucan may be involved in cell wall loosening and elongation. Auxin has been found to promote the release of a water soluble xyloglucan from its bound state in the cell wall. [24] A decrease in xyloglucan molecular weight has been shown to take place during auxin-induced cell wall extension. [25] Xyloglucan fragments have been observed to inhibit auxin-induced cell wall elongation. [26] Thus, the evidence suggests that xyloglucan plays some critical role in the auxin-induced process for cell wall extension. However, at present, researchers cannot determine the exact mechanism.

## POLYMER ADSORPTION

The adsorption of polymers at solid-liquid interfaces is a phenomenon that has generated considerable interest both academically and industrially over the years. For the papermaker, adsorption of polymers onto cellulose surfaces has long been a means of extending the useful range of his product. Reviews have been published [27,28,29] showing a number of qualitative, or sometimes semi-quantitative, trends that can be observed for most polymer adsorption systems. But, as Kindler [30] pointed out, "Nearly any statement concerning the behavior of polymer adsorption may be contradicted by results obtained with some specific system." And so, the following statements serve as general trends not as absolutes for polymer adsorption.

Polymer adsorption isotherms (see Figure 5) can be drawn to show the relationship between the amount of polymer adsorbed per unit surface area versus the polymer concentration at equilibrium. Usually a sharp initial rise in adsorption

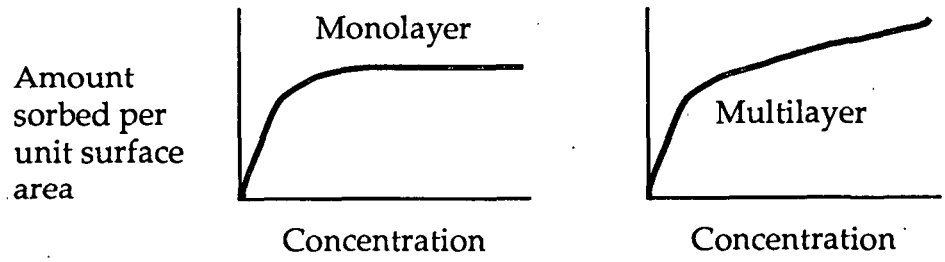


Figure 5. Adsorption isotherms illustrating monolayer and multilayer adsorption.

is followed either by a plateau indicating monolayer sorption or a continually increasing region (multilayer sorption).

Only a fraction of the segments in the adsorbed polymer are actually in contact with the surface of the adsorbent (Figure 6). The remaining segments are looped into the solution phase. Three types of segment sequences are recognized: trains, loops, and tails. The actual configuration of an adsorbed polymer molecule is difficult to determine; the distribution of the lengths of loops, trains, and tails is governed by such factors as solvent quality, molecular weight, and polymer/surface interaction energy.

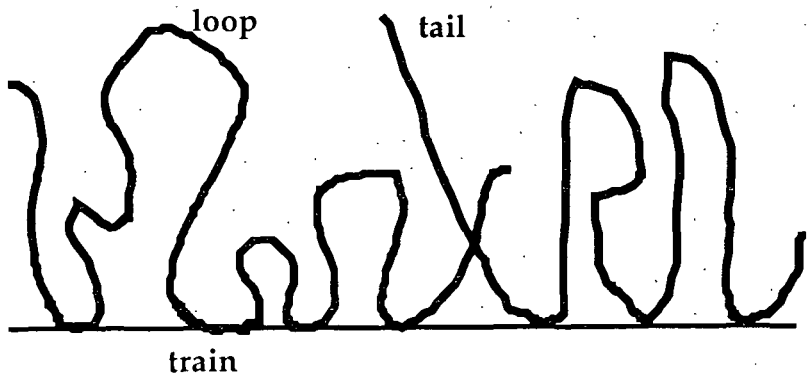


Figure 6. Adsorbed polymer molecules with loops, trains, and tails.

Adsorption of a polymer, which has a wide molecular weight distribution, often follows a predictable pattern. The smaller, more mobile, low molecular weight molecules are initially adsorbed from the polymer solution. These lower molecular weight molecules are later displaced by the less soluble, more slowly diffusing, higher molecular weight molecules. The adsorption of the latter is thermodynamically favored.

For a long time, lack of polymer desorption upon dilution with solvent was regarded as proof that the adsorption was irreversible. Currently it is believed that a dynamic equilibrium exists wherein sections of the adsorbed polymer are constantly attaching to and detaching from the adsorbent. With higher molecular weight polymers, the difficulty with total desorption of the polymer appears to be the statistical improbability of removing all of the "trains" at the same time. Therefore, an apparent irreversibility of polymer adsorption was hypothesized.

In 1980, Cohen Stuart, Scheutjens, and Fleer [31] showed that this apparent polymer adsorption irreversibility was not necessarily true irreversibility. They found that dilution of the system with solvent lowers the concentration of the smaller molecules that were already in solution. The distribution of the adsorbed higher molecular weight and smaller molecules in solution remained unchanged. Therefore, the only effect dilution had was to shift the surface to volume ratio to a lower value without changing the adsorbed amount of polymer. Thus, Cohen Stuart *et al.* [31] concluded that experimental evidence of no desorption was not sufficient proof for the irreversibility of polymer adsorption.

The effect of temperature on polymer adsorption is usually either small or absent. Depending upon the polymer/solvent system chosen, adsorption can either

increase or decrease with increasing temperature. Solvent power is a function of temperature.

The amount of polymer adsorbed is very sensitive to 1) solvent choice, 2) polymer composition, and 3) adsorbent. Adsorption increases with decreasing solvent quality; thus, less polymer is adsorbed from a good solvent than a poor one. The chemical constitution of a polymer, including functional groups and branching, plays an important role in determining the amount of polymer adsorbed and the adsorption isotherm shape. And, the substrate onto which the polymer adsorbs can also influence the adsorption process. For a cellulose adsorbent, surface area and carboxyl content can affect polymer adsorption.

## SORPTION OF OTHER HEMICELLULOSES

In an early hemicellulose sorption study, Most [32] sorbed $^{14}C$ labeled slash pine hemicellulose fractions (isolated with 1, 4, 7, and 16% potassium hydroxide) onto an alpha cellulose pulp. He found: 1) that his hemicellulose fractions were retained by adsorption, 2) the rate of sorption slowed after a monolayer was formed, and 3) the amount of hemicellulose sorbed was dependent upon the initial hemicellulose concentration added. After a monolayer of hemicellulose is sorbed, further hemicellulose sorption takes place to form a multilayer. Increased sorption and strength improving properties of handsheets were observed with those fractions having a higher mannose content.

Eriksson, Samuelson, and Viale [33] studied the sorption of a galacto-glucomannan (GGM) isolated from sulfite cooking liquors onto cotton. They found increased hemicellulose adsorption at higher temperatures and little affect due to pH in the range between pH 2 and 7. From their graph of adsorption vs. final

hemicellulose concentration, multilayer adsorption can be ascertained. When the cotton linters with the sorbed GGM were washed with water, no GGM desorbed and the adsorption was believed irreversible. Almost total desorption of the polymer occurred upon treatment with 10% sodium hydroxide, however.

Laffend [34] also studied the sorption of a glucomannan (isolated from *Tubera salep*) onto cotton linters. He examined the effect of removing functional groups on this polymer's sorption (native softwood glucomannan contains acetyl groups). Deacetylated glucomannan, when compared with the native polymer, was found to have 1) a higher rate of sorption onto cotton linters; 2) a higher specific sorption for a given amount of glucomannan addition, and 3) multilayer sorption. In contrast, monolayer sorption was found for the acetylated glucomannan. The sorption of the acetylated polymer reached a plateau value (≈7.5%), but deacetylated glucomannan adsorbed in ever increasing amounts as its initial concentration was increased.

Clayton and Phelps [35] published a study on the sorption of spruce glucomannan and birch xylan onto alpha cellulose wood fibers in 0.02N alkali. The xylan was less readily adsorbed than the glucomannan; they ascribed this to the presence of uronic acid carboxylic groups on the xylan. From their calculations on average activation energies from the rates of sorption, they were able to hypothesize that the sorption was of the physical or van der Waals type.

Walker [36] studied the sorption of reduced and unreduced spruce 4-O-methyl-glucuronoarabinoxylan onto cotton linters. He found that partial reduction of the hemicellulose uronic acids to alcohols caused a significant increase in hemicellulose adsorption. A preferential, but slower, sorption of higher molecular weight species was also observed. [36]

Another class of polysaccharides whose sorption properties have been studied extensively is the gums. Sorption of locust bean gum, guar gum, etc. has been examined by the papermaker because gum addition can advance beating (e.g., lower amount of beating needed to reach a certain pulp freeness) and higher paper strength properties may be achieved. Dugal and Swanson [37] reviewed theories on the mechanism of gum adsorption and the beneficial effects of these beater adhesives, in their paper on guar gum (a galactomannan containing 3 mannose: 2 galactose). Russo [38] examined the sorption of locust bean gum (a galactomannan containing 3-4 mannose: 1 galactose) onto a bleached sulfite pulp. Multilayer sorption was found. As was expected, increased locust bean gum sorption (both amount and rate) with increasing hydrodynamic specific surface area of the fibers was reported.

In summary, different hemicellulose polymers can be seen to have different sorption properties. The amount of sorption and whether a multilayer or monolayer forms can vary considerably. The presence or absence of functional groups on a given polymer can play an important role in its sorption behavior.

## THESIS OBJECTIVE

Prior to this thesis, only Valent and Albersheim's study [18] on xyloglucan fragment sorption onto cellulose powder had investigated the xyloglucan:cellulose relationship. Their study incorporated unusual conditions; seven- and nine-sugar fragments (not intact polymer), cellulose powder (not fibers), and organic solvents (nonaqueous environment) were employed. The purpose of this thesis was, therefore, to study the sorption of the xyloglucan polymer isolated from *Tamarindus indica* onto cellulose fibers in an aqueous environment including a

comparison of this sorption behavior with that of other hemicelluloses and polymers.

This study was to lay the groundwork for future experiments concerning xyloglucan in the plant cell wall. These future investigations would include the effect of xyloglucan on 1) the formation of cellulosic microfibrils; this study is presently in progress at The Institute of Paper Chemistry, 2) the expansion of the cell wall during growth, and 3) tissue cultured cells themselves during periods of growth.

## EXPERIMENTAL PLAN

The experimental plan for this thesis was broken down into three main sections: isolation, characterization, and sorption. First, the xyloglucan polymer was to be isolated and purified in a sufficient quantity to complete the objectives of the thesis. Special efforts were to be made to retain the chemical and physical structure of the native polymer (i.e., no removal of acetyl groups).

Characterization of this xyloglucan was then required. Structural elucidation was to include sugar analysis and methylation data. Four functional groups were to be quantified: acetyl, pyruvate, methoxyl, and carboxyl groups. Finally, the molecular weight distribution would be determined.

The xyloglucan sorption onto cellulose fibers was then to include two sets of experiments. The first set of sorption experiments was to determine the conditions necessary for a practical run (i.e., xyloglucan concentration, pulp consistency, time to equilibrium, and reproducibility within a run). The second set of sorption experiments was to 1) define the equilibrium adsorption isotherm, 2) determine the

effect of removing any natural functional groups from the xyloglucan polymer and 3) evaluate the effect of xyloglucan molecular weight (MW), using narrow MW fractions. Laffend [34] had previously found that removal of acetyl substituents from a glucomannan created a large difference in its sorption properties.

# EXPERIMENTAL

## XYLOGLUCAN ISOLATION AND PURIFICATION

Xyloglucan was extracted from the ground, decorticated and defatted seeds (10 g) of *Tamarindus indica* with boiling distilled water (1 L) for 30-45 min. [2] The suspension was filtered through coarse filter paper and then a layer of diatomaceous earth (Manville Celite Filter Aid). Ethanol (95%, 600 mL) was added to this clear filtrate to form a gelatinous precipitate. This was drained on cheesecloth and squeezed out to remove as much alcoholic liquor as possible. The fibrous mass was stirred with 50% ethanol (100 mL), drained, and squeezed on cheesecloth. This product was either freeze dried to yield the crude xyloglucan (35% yield) or further purified.

To purify the xyloglucan, crude product was dissolved in distilled water (1 L) and dilute Fehling's solution (20 mL in 130 mL distilled water) was added with vigorous stirring. The viscous blue gel that formed was drained on cheesecloth and washed with distilled water (3 x 500 mL). The gel was suspended in distilled water (600 mL) and dissolved by adding hydrochloric acid (4 mL, 2N). The xyloglucan was precipitated with ethanol (95%, 400 mL), drained, squeezed on cheesecloth, and washed with 50% ethanol (100 mL). Ethanol was removed *in vacuo* and water was periodically added to maintain the xyloglucan as a non-viscous solution. The product was freeze dried to give purified xyloglucan.

## XYLOGLUCAN CHARACTERIZATION

### Nitrogen Analysis

Midwest Micro-Labs, LTD (Indianapolis, IN) performed the nitrogen analyses

using an elemental analyzer.

Carbohydrate and Ash Determinations

A. Webb of the I.P.C. Analytical Department conducted the carbohydrate analyses using the method of Borchardt and Piper. [39] Ash content was also determined by the I.P.C. Analytical Department using Tappi Standard T211 cm-80. [40]

Methylation Analysis

The methylation analysis was performed by the methylation and trifluoroacetic acid hydrolysis procedures described by Nealey. [16] Xyloglucan (100 mg) was dissolved in dry dimethyl sulfoxide (10 mL). Finely powdered sodium hydroxide (400 mg) and methyl iodide (2 mL) were stirred with the xyloglucan solution for twelve min. [41] Water (40-50 mL) and chloroform (20 mL) were added to the reaction mixture and the extracted chloroform layer was washed with water (3 x 40-50 mL). The chloroform layer was concentrated *in vacuo* (40°C) to dryness in a serum bottle. Acid hydrolysis was accomplished by the addition of trifluoroacetic acid (2N, 10 mL) [42] to the residue; the mixture was heated at 120°C for 2 h. The trifluoroacetic acid was removed *in vacuo* at 40°C. Sodium borohydride (10 mg) and water (10 mL) were added (room temperature, overnight) to reduce the methylated monomers. Glacial acetic acid was used to quench the borohydride and then removed by concentration *in vacuo* below 40°C with repeated methanol additions. The sample was then acetylated with acetic anhydride (4 mL, 120°C, 3 h). The sample was allowed to cool prior to dilution with water and then extracted with chloroform to separate the sample from the sodium salts. The chloroform extract was concentrated *in vacuo* with addition of methanol until the acetic anhydride was removed. This sample was dissolved in dichloromethane prior to

analysis by gas liquid chromatography (GC) or gas liquid chromatography/ mass spectroscopy (GC/MS). GC analyses of the methylation products were run isothermally on a Packard Model 417. A 2 mm (ID) x 6 ft glass column packed with SP-2340 (Supelco, Inc.) was used at 170°C. The GC/MS analyses were run by L. Borchardt on a Hewlett-Packard 5985 system using a similar column. The temperature program was a 13 min hold at 170°C followed by a 3°C increase per min to 220°C.

Functional Group Analysis

Methoxyl contents were determined by the Microanalytical Laboratory of the University of Vienna, Waehringerstrasse 38, A-1090 Vienna, Austria. Acetyl and pyruvate group identification were done by G. Yates of the I.P.C. Analytical Department using a $^1$H-NMR technique described by Kennedy *et al.* [43] Total carboxyl content was determined by the method of Whistler and Feather. [44]

Molecular Weight Distribution Determination Using SEC

A 2.5 cm (ID) x 100 cm size exclusion chromatography (SEC) column of Sepharose CL-4B (Pharmacia Fine Chemicals) was used for the molecular weight (MW) distribution determination. Eluant (0.1N NaOH) was fed to the column bed top by siphon and effluent was pumped out (60 mL/h) by a WIZ peristaltic pump (ISCO). Fractions (5 mL/tube) were collected using a Retriever III Fraction Collector (ISCO) and later analyzed.

Calibration of the column involved the use of a series of Dextran MW markers (Pharmacia): T2000 ≈ 2,000,000 MW, T500 = 488,000, T150 ≈ 150,000, T70 = 73,400 MW, T40 = 35,600 MW. Approximately 5 mg of the Dextran MW marker was

dissolved in 0.1N NaOH (0.5 mL) and directly applied to the SEC column bed top. After the fractions were collected, total carbohydrate analysis was performed by the phenol-sulfuric acid method. [45] Elution volumes for the MW markers were calculated as chromatogram peak values (carbohydrate concentration vs. fraction number). The logarithm of the MW of each marker vs. its elution volume was plotted to obtain the calibration curve for the SEC column.

Xyloglucan (2-22 mg) was combined with 0.1N NaOH (0.5-2 mL) and refrigerated overnight to aid dissolution. The next morning the sample was applied to the SEC column. The column was run under the same conditions as used with the MW markers. Samples (1 mL) were slightly acidified (0.15 mL 1N $H_2SO_4$) and analyzed by Kooiman's iodine/potassium iodide quantitative method [2] for xyloglucan analysis.

One set of xyloglucan fractions from the SEC column was analyzed by both the phenol-sulfuric acid method [45] for total carbohydrate measurement and Kooiman's procedure. [2] This experiment was used to determine whether the differing molecular weights of this xyloglucan affected its measurement by Kooiman's procedure. Xyloglucan is believed to entrap the iodine molecules within its structure [46], and thus, differing xyloglucan MWs could affect this entrapment. No significant difference was seen in the results from these two quantitative methods.

Molecular Weight Determination Using GPC/LALLS

Bill Mahn of Milton Roy performed molecular weight analysis of the xyloglucan and its fractions using a gel permeation chromatography/low angle laser light scattering (GPC/LALLS) system.

Phenol-Sulfuric Acid Method for Total Carbohydrate Measurement [45]

Sugar solution (1 mL), containing between 0.1-0.7 mg of sugar, was pipetted into a spectrophotometric tube. A 5% (w/v) aqueous solution of phenol (1 mL) and concentrated sulfuric acid (5 mL) were added. The stream of acid was directed against the liquid surface in order to gain good mixing. The tubes were allowed to stand at room temperature (10 min), shaken, and allowed to cool (10-20 min) prior to insertion into a Bausch and Lomb Spectronic 20 spectrophotometer. Percent transmittance was read at 490 nm. Samples were run in triplicate to minimize error. Standard concentration curves (concentration vs. absorbance) using Dextran T10 and T500 were prepared and the results from the linear regression of the data evaluated using Student's t test. All values from both molecular weight markers were within the 95% confidence limits.

Quantitative Xyloglucan Determination [2]

Xyloglucan solution (1 mL), containing 0.01-0.30 mg of xyloglucan, was combined in a spectrophotometric tube with 5 mL of iodine/potassium iodide solution (0.5 g iodine and 1 g potassium iodide with enough distilled water to make 100 mL) and 5 mL of sodium sulphate solution (20 g sodium sulphate diluted with distilled water to give 100 mL). The samples were well mixed by shaking, allowed to stand at room temperature (60 min), and then read in a Bausch and Lomb Spectronic 20 spectrophotometer at 660 nm. Samples were run in triplicate to minimize errors. Percent transmittance was read and the absorbance calculated. A standard concentration curve (concentration vs. absorbance) was used to calculate the xyloglucan concentration from the absorbance. Linear regression analysis of the standard concentration curve confirmed the linearity of the graph ($R^2 \sim 0.996$).

## COTTON LINTERS PREPARATION AND CHARACTERIZATION

Cotton linters (HSR grade) were obtained from Alpha Cellulose Corporation. The linters (500 mg) were extracted (1.5 L 95% ethanol: 1.5 L chloroform, 48 hours) to remove any residual oils or extractives by H. Corbett using the method of McCarthy. [47] This pulp was not, however, easily dispersible in water. Freezing followed by solvent exchange (chilled 95% ethanol, then room temperature 95% ethanol) of the cotton linters pulp, as described by Laffend [34], yielded a pulp easily dispersible in water. The linters were stored in a conditioned room (73°F, 50% RH) prior to use so that their moisture content remained constant.

Carbohydrate analysis [39] was performed on the cotton linters by A. Webb of the I.P.C. Analytical Department. Glucose was the only sugar found in an appreciable amount (98%). Fiber length distribution was run on the Kajaani FS-100 by the I.P.C. Fiber Identification group. The length weighted average of the linters was 0.97 mm. Measurement of the specific surface area of the cotton linters was done by two methods. B. Andrews and K. Rankin used filtration resistance [48] to examine the hydrodynamic specific surface area (area exposed to fluid drag; $1.06 \times 10^4$ $cm^2/g$) and the specific volume denied to flow (2.21 cc/g). The $N_2$ adsorption [49] specific surface area ($1.7 \times 10^4$ $cm^2/g$) determined by N. Colson, measures the surface accessible to the gas (this would include the lumen and internal pores). This should be larger than that found by filtration resistance. The hydrodynamic specific surface area was used for calculating the area each molecule occupies after sorption and for comparisons with other polymers sorbed onto cellulose surfaces. This specific surface area was chosen because it gives the more probable, accessible surface area for the xyloglucan polymer.

## SORPTION STUDIES

### Sorption Apparatus

All sorption runs were carried out in sealed centrifuge tubes (50 mL) that were rotated in a constant temperature bath (25 ± 0.5°C). The tubes were attached to the peripheries of two parallel, notched metal disks that were mounted on a common shaft as described previously. [30] As the shaft rotated (15 rpm), gentle agitation was provided to the sorption tube contents.

### Sorption Procedure

The sorption experiments of the xyloglucan onto the cellulose fibers followed a set procedure. Cotton linters (0.16 g o.d.) were weighed and distilled water was pipetted directly into the sorption tubes in the first sets of Sorption Experiments (SE#1-3D). The tubes were then sealed, shaken by hand until the linters were thoroughly dispersed, and placed on the agitator in the constant temperature bath overnight.

A xyloglucan stock solution was prepared in a volumetric flask by dissolving a known amount of xyloglucan (dried at room temperature over phosphorous pentoxide) in distilled water. Dissolution was aided by refrigerating the solution overnight. Gentle agitation completed dissolution. Aqueous xyloglucan solutions were found to remain stable for three weeks. However, all xyloglucan solutions and samples were made up as close to the time of use or analysis as possible.

The xyloglucan stock solution was also placed in the constant temperature bath overnight prior to the sorption experiments. The next day xyloglucan stock solution was pipetted into the tubes via an automatic pipette and the tubes were

returned to the agitator. At specified times, a tube was removed from the bath and the supernatant isolated by quickly filtering the suspensions through a coarse sintered glass filter funnel. During the final sorption experiments (SE#3E-4), the distilled water and xyloglucan stock solution were weighed, via an analytical balance, directly into the sorption tubes to eliminate possible error associated with using automatic pipettes.

The amount of xyloglucan sorbed was determined by measuring the changes in supernatant concentration (the initial xyloglucan concentration, $C_i$, minus the final or equilibrium concentration, $C_e$). Initial xyloglucan concentration was measured using control sorption tubes containing only distilled water and xyloglucan solution. Cotton linters control tubes (cotton linters plus water, no xyloglucan) were also run. No xyloglucan was ever found in these tubes. Xyloglucan concentrations were obtained using Kooiman's iodine/potassium iodide method. [2] The specific sorption ($\Gamma$) was calculated as the amount of xyloglucan adsorbed ($C_i$ - $C_e$) per gram of cotton linters used.

Isolation of Narrow MW Fractions

Narrow MW fractions were isolated using the SEC column. Three adjacent tubes (5 mL/tube) were combined to make up each narrow MW fraction (15 mL/fraction). The fractions were neutralized (pH $\approx$ 7) using hydrochloric acid (1.5 mL, 0.1N). Sodium and chloride ions were removed via diafiltration (Amicon 202, YM10 membrane, 50 psi). Diafiltration was halted when the conductivity of the filtrate matched that of distilled water. The samples were freeze dried and the process repeated until enough of each fraction was obtained for the sorption runs.

# RESULTS AND DISCUSSION

## XYLOGLUCAN ISOLATION AND PURIFICATION

Xyloglucan polymer was isolated from ground, decorticated *Tamarindus indica* seeds (which had been in storage for twenty years) using a modified procedure of Kooiman. [2] Hot water extraction of the tamarind seed powder was followed by precipitation of the crude xyloglucan with ethanol. Traditionally, purification of crude xyloglucan was then required. Rao [50] reported that this product normally contains about 14% associated proteins. The formation of a polysaccharide-metallic complex by the addition of metallic hydroxide or alkaline salt solutions (i.e., Fehling's) has been used to remove these proteins. This purification procedure would, however, subject the xyloglucan to possible alkaline oxidative cleavage (such as the peeling reaction referred to in pulping studies [51]) and alkaline removal of potential functional groups (i.e., acetyl groups) [51].

Fortunately, the isolated crude xyloglucan did not contain any significant amounts of associated proteins (<0.1% nitrogen). The protein was believed to have been denatured and, subsequently, removed during the isolation procedure. Therefore, no purification was necessary. From this observation, questions arose concerning the possible degradation of the polymer due to aging (over 20 years old). Fresh tamarind seeds were obtained from Dr. H. S. Dugal, processed to yield a crude xyloglucan, and analyzed. See Appendix I for details. A small amount of protein (1.75%) was found in the "crude" xyloglucan isolated from these fresh tamarind seeds. No discernible differences between this xyloglucan and the twenty year old tamarind seed powder xyloglucan, however, were apparent. The crude xyloglucan from the 20 year old source was then isolated in quantity (approximately 70 grams

total) and utilized during the remainder of this thesis.

## XYLOGLUCAN CHARACTERIZATION

Characterization of the isolated xyloglucan identified 96-97% of the components (Table 6). Characterization was divided into three main sections. First, structural elucidation was performed to resolve whether the isolated xyloglucan was similar in structural subunits to those found in literature. Second, functional group analysis was done to ascertain the presence and quantity of any functional groups. Third, the molecular weight distribution was determined.

Table 6. Chemical analysis of xyloglucan samples from two different batches.

| | Batch 3636-67-1 Scaleup 2 | Batch 3636-67-2 Scaleup 3 |
|---|---|---|
| Nitrogen Determination, % | <0.1 | <0.1 |
| Sugar Analysis | | |
| Araban, % | 1.4 | 1.4 |
| Xylan, % | 28.4 | 28.8 |
| Mannan, % | 1.1 | 1.5 |
| Galactan, % | 14.6 | 15.4 |
| Glucan, % | 50.3 | 49.8 |
| Total Carbohydrate, % | 95.8 | 96.9 |
| Ash, % | 0.19 | 0.22 |

Structural Elucidation

Several structures [2,3,52] have been proposed for the xyloglucan isolated from *Tamarindus indica*. All consist of a β-(1→4)-linked glucan backbone with side chains containing xylosyl and galactosyl sugars. Xyloglucan structural elucidation included sugar analysis (Table 6) and a methylation study. The sugar analysis revealed a sugar ratio (4.00 glucose: 2.37 xylose: 1.16 galactose) similar to those reported in literature (Table 7).

Table 7. Sugar ratios of various xyloglucans isolated from *Tamarindus indica*.

| | Sugars | | |
|---|---|---|---|
| Reference | Glucose | Xylose | Galactose |
| This thesis | 4.0 | 2.4 | 1.2 |
| Kooiman [2] | 4.0 | 3.0 | 1.3 |
| Srivastava and Singh [3] | 4 | 2 | 1 |
| Reis *et al.* [52] | 4 | 2.7 | 1.3 |

Methylation analysis is a widely accepted method for determining the substitution of the carbohydrate monomeric units. One step permethylation methods, advanced gas liquid chromatography (GC), and gas liquid chromatography/mass spectroscopy (GC/MS) have made methylation analysis one of the most prominent methods for polysaccharide characterization. The Hakamori method [53] which utilizes sodium hydride, dimethylsulfoxide, and methyl iodide, is the most widely used permethylation procedure. Sodium methylsulfinylmethanide in dimethylsulfoxide is prepared to form the dimsyl anion ($CH_3SOCH_2^-$).

Unfortunately, this procedure is both tedious and extremely water sensitive. In 1984, Ciucanu and Kerek [41] reported that the hydroxide anion (formed from sodium hydroxide in dimethylsulfoxide) ionizes carbohydrate hydroxyls more rapidly and completely than the dimsyl anion. Their method, although still sensitive to water, is much simpler to use and easier to maintain in a water-free atmosphere. Therefore, Ciucanu and Kerek's [41] method was employed to methylate the xyloglucan.

After polysaccharide methylation, the polymer is hydrolyzed, reduced, and acetylated. The partially methylated alditol acetates were then analyzed by GC and GC/MS. Considerable work has been done on identifying these sugars. [54,55,56] Jansson *et al.* [56] have published a mass spectra library of methylated sugar alditol acetates. Identification of the methylated alditol acetates from xyloglucan reveals its branching pattern. Methylation analysis does not, however, provide any information on monomer sequence or anomeric state of the monomers.

Nealey [16] did considerable work on the methylation and characterization of his xyloglucan. He determined the retention times (RT) for his fragments and for methylated alditol acetates from standard sugars on both ECNSS-M and SP-2340 columns under isothermal GC conditions. Known sugar retention times were graphed against literature values [54] to produce calibration curves. The GC column was calibrated daily against standards to provide exact correlation between the retention times of the xyloglucan and literature values. Some xyloglucan fragment peaks (Figure 7) were readily identifiable using retention times, the known carbohydrate composition, and reported xyloglucan structures. [16,17,57] Unfortunately, some retention times were fairly close together and did not precisely fall on any specific literature value. Further identification was done by combining the GC and

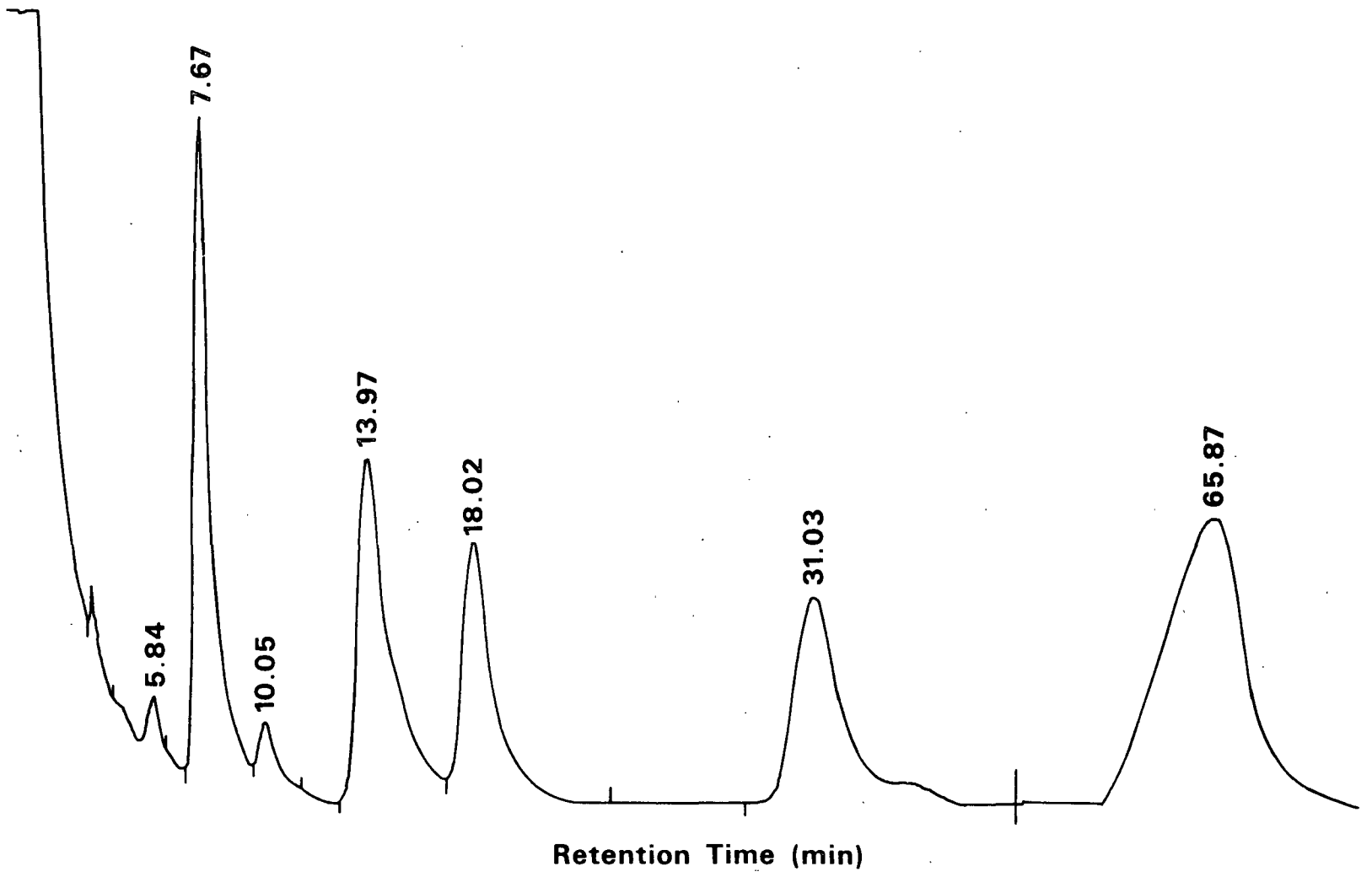


Figure 7. Gas chromatogram of the partially methylated alditol acetates from xyloglucan (36363-67-1). Isothermal run at 170°C on SP-2340.

GC/MS results. Appendix II contains the total ion chromatogram of the xyloglucan methylated alditol acetates and the mass spectrum of each peak. The mass spectra of partially methylated alditol acetates are not greatly different from one another [56] and only compound class (i.e., methylated pentose or hexose) can be determined. Furthermore, since the GC retention times can be inconclusive, a combination of the results from both methods allows a more positive identification to be made. Table 8 illustrates how the identification is deduced; species identified by GC RT are compared with the compound class determined by MS to give the deduced species. The peak at 5.84 min was believed to be due to 235 Ara (abbreviations defined at end of Table 8) and not 234 Fuc, a 6 deoxy 234 Hexose, because no fucose was ever identified during the carbohydrate analyses.

*Tamarindus indica* xyloglucan had previously been characterized by methylation analysis. [2,3,4,58] Srivastava and Singh [3] collected and reported the data from these studies; Table 9 compares this thesis' results with the literature values. The same five major methylated monomeric units were found in all studies. Srivastava and Singh [3] also reported the presence of 235 Ara ; a small quantity of 235 Ara was also identified in this thesis. The relative ratio or amount of each sugar found varied with the individual study, but similarities between the literature and this thesis can be seen. From Table 9, a good correlation between the normalized amount of terminal sugars (234 Xyl, 2346 Gal, and 235 Ara; 2.04) and C-6 substituted glucose found in this thesis (23 Glu; 2.3) was demonstrated.

Functional Group Analysis

It was originally believed that some functional groups (e.g., acetyl, pyruvate, methoxyl, and carboxyl) might be present on *Tamarindus indica* xyloglucan.

Table 8. GC and GC/MS data on the alditol acetates of a methylated xyloglucan (3636-67-1). Isothermal run at 170°C on SP-2340.

| Retention Time (RT), min | Area, % | Possible Species from: RT | MS | Deduced Species |
|---|---|---|---|---|
| 5.84 | 0.563 | *235 or 234 Fuc/ 235 Ara/ 234 Xyl | 235 Pentose/ 6 deoxy 234 Hexose | 235 Ara |
| 7.67 | 15.805 | 234 Xyl/ 234 Ara/ 1346 Gal or Man/ 234 Fuc | 234 Pentose | 234 Xyl/ 234 Ara |
| 10.05 | 1.614 | 2346 Glu or Man/ 35 Ara/ 34, 23, or 24 Rha | 35 Pentose | 35 Ara |
| 13.97 | 16.527 | 2346 Gal/ 23 Fuc | 2346 Hexose | 2346 Gal |
| 18.02 | 12.756 | 23 or 34 Xyl/ 2 Rha | 23 or 34 Pentose | 23 or 34 Xyl |
| 31.03 | 15.812 | 236 Glu/ 346 Gal/ 234 Glu or Man | 236 Hexose | 236 Glu |
| 65.87 | 36.924 | 24 Glu or Man/ 23 Glu/ 346 Glu or Man/ 6 Gal | 23 Hexose | 23 Glu |

*The numbers given indicate the position of the methyl groups on the alditol acetate, i.e., 235 Ara = 2,3,5-tri-O-methyl 1,4-di-O-acetyl arabinitol. Sugar abbreviations: Ara = Arabinose, Fuc = Fucose, Gal = Galactose, Glu = Glucose, Man = Mannose, Rha = Rhamnose, and Xyl = Xylose.

Table 9. Sugars obtained upon hydrolysis of methylated xyloglucan from *Tamarindus indica* seed powder.

| Sugar | Normalized Relative Ratio | | | | |
|---|---|---|---|---|---|
| | *A | B | C | D | E |
| **234 Xyl | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 2346 Gal | 1.0 | 0.7 | 0.5 | 1.0 | 1.0 |
| 23/34 Xyl | 0.8 | 0.3 | 0.5 | 0.8 | 0.9 |
| 236 Glu | 1.0 | 0.7 | 0.5 | 0.8 | 0.9 |
| 23 Glu | 2.3 | 2.0 | 1.5 | 1.8 | 1.7 |
| 235 Ara | 0.04 | 0.3 | -- | -- | -- |

*A = data from this thesis; B = reference [3]; C = reference [2]; D = reference [4]; E = reference [58].
**See Table 8 for definitions of these abbreviations.

Albersheim [59,60] reported finding O-acetyl substituents on *Acer pseudoplatanus* extracellular xyloglucan. Deacetylation of glucomannans has been shown to create large differences in its sorption onto cellulose fibers. [34] Therefore, the presence of functional groups needed to be determined. If any functional groups were present, sorption experiments were to be performed with and without these groups. No significant amount of any functional group was found on the xyloglucan (Table 10). Consequently, no comparison between the sorption of substituted and unsubstituted xyloglucan could be performed, and it was deemed unnecessary to artificially place such substituents on the native polymer.

Molecular Weight Distribution

Considerable variation in xyloglucan molecular weight has been reported in

Table 10. Functional groups present in *Tamarindus indica* xyloglucan.

| Functional Groups | Batch 3636-67-1 | Batch 36363-67-2 |
|---|---|---|
| Carboxyl | NGL* | NGL |
| Acetyl | NGL | NGL |
| Pyruvate | NGL | NGL |
| Methoxyl, wt % | 0.21 | 0.44 |

*NGL = Negligible

the literature. Values ranging from 7,600 for *Acer pseudoplatanus* cell wall xyloglucan [17] to 330,000 for the xyloglucan isolated from pea stems [11] have been given. Reported tamarind xyloglucan molecular weights include 10,500 [6] and 115,000 [2]. A review of recent molecular weight determination techniques led to the use of size exclusion chromatography (SEC, also called gel filtration or gel permeation chromatography) for determining the xyloglucan's molecular weight distribution.

Size exclusion chromatography is a liquid column chromatographic method for separating molecules according to their size. The sample is introduced onto a column filled with a gel or other porous material (called the stationary phase). The column is eluted with the same solvent used to dissolve the sample. The actual separation of the sample molecules is dependent upon each molecule's ability to enter the pores of the stationary phase. The largest molecules, which do not enter any of the pores, move through the chromatographic bed the most quickly. Small molecules which diffuse into the gel pores, move more slowly through the column. Therefore, molecules are eluted in order of decreasing molecular size.

The molecular weight distribution of a sample can be determined by size

exclusion chromatography, providing there are no shape variations. Column calibration is performed using a series of compounds of similar molecular shape, structure, and known molecular weight. These molecular weight standards or markers are eluted through the column to determine their elution volumes ($V_e$ = volume of column effluent that corresponds to peak maximum). The linear relationship between the logarithm of the molecular weight of these markers and their elution volumes is determined. From this calibration curve (Figure 8; see Appendix III for individual chromatograms of each molecular weight marker), the molecular weight number and weight averages along with information on the molecular weight distribution of polymers passed through the column can be calculated. The molecular weight distribution curve for the isolated xyloglucan is shown in Figure 9 (data in Appendix III). Accurate number and weight average molecular weights could not be estimated, because the exact molecular weight of Dextran T2000 (≈2,000,000) was not known. The approximate molecular weight at the SEC chromatogram peak is $1.4 \times 10^6$. This determination assumes that the size or volume of a xyloglucan molecule of given molecular weight in solution is equal to that of a dextran molecule of the same molecular weight in solution. To confirm the SEC molecular weight, gel permeation/low angle laser light scattering (GPC/LALLS) analysis of the xyloglucan polymer was performed. A peak molecular weight value of $1.2 \times 10^6$ was obtained by this method.

## SORPTION EXPERIMENTS

### Time to Equilibrium and Reproducibility

Xyloglucan sorption onto cellulose fibers at 25°C was followed with respect to time to determine when equilibrium was reached (Figure 10). At approximately 24

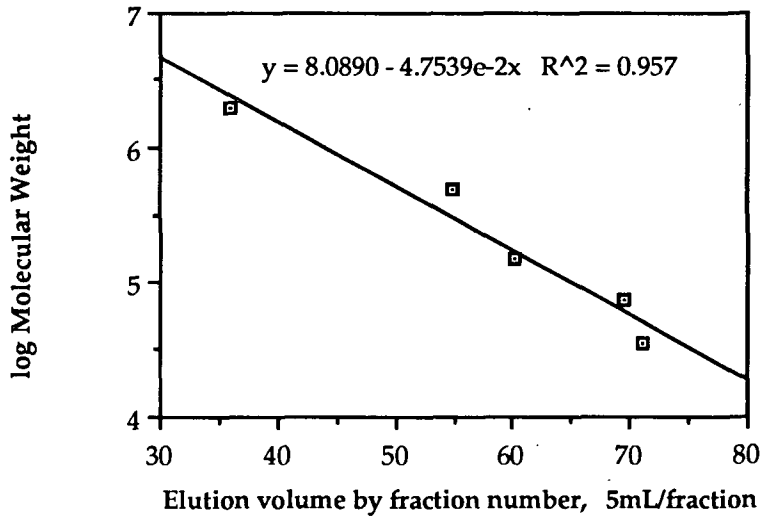


Figure 8. Calibration curve of dextran molecular weight markers on Sepharose CL-4B, 0.1N NaOH eluant at 60 mL/h, 5 mL/fraction collected.

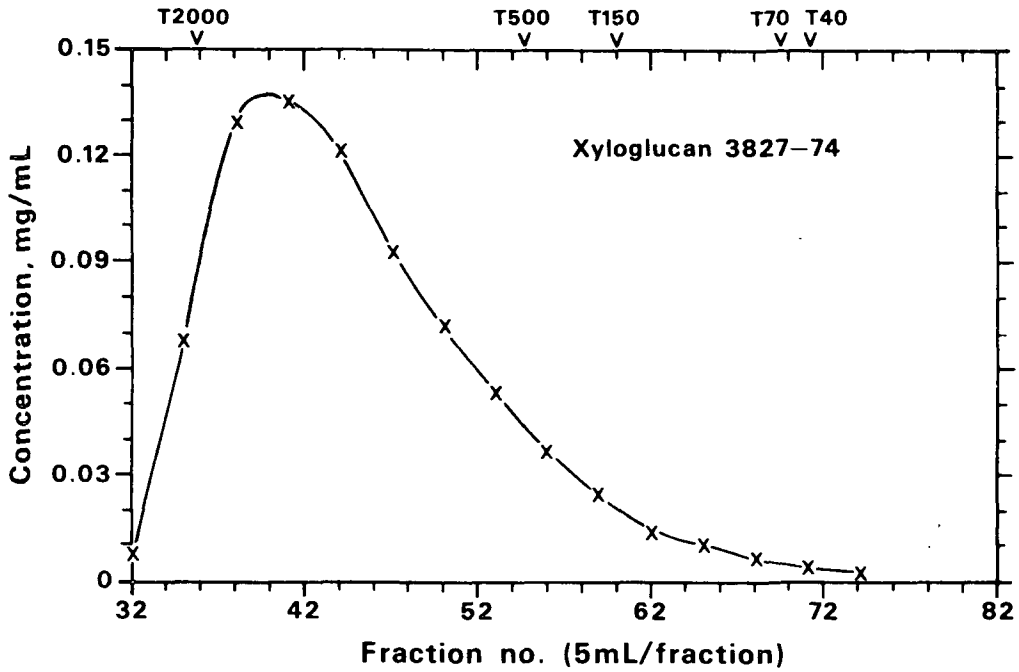


Figure 9. Size exclusion chromatogram of xyloglucan isolated from *Tamarindus indica*. Sepharose CL-4B, 0.1N NaOH eluant at 60 mL/h, 5 mL/fraction collected.

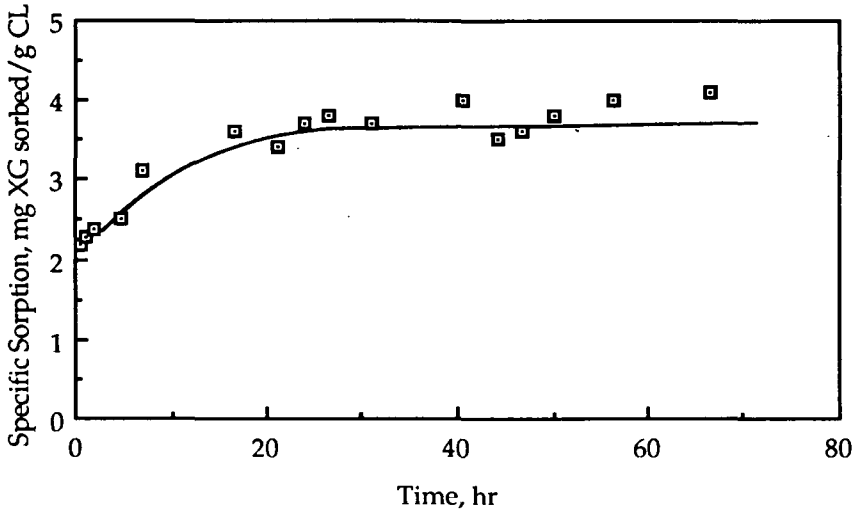


Figure 10. Time to sorption equilibrium. Sorption Experiment#2B, 3827-20. Initial concentration = 0.0867 mg/mL.

hours, but definitely before 48 hours, a plateauing of the graph illustrated that equilibrium had been attained. (See Appendix IV for data from the Sorption Experiments.) This equilibrium was much slower than the pea xyloglucan sorption experiments reported by Hayashi *et al.* [22] in which equilibrium was attained within 4 hours at 40°C. This time difference to equilibrium could be due to the larger molecular weight and lower temperature used in this thesis or different concentrations of xyloglucan and cellulose surface. Subsequent experiments were run 48 hours to guarantee equilibrium sorption.

To determine whether sorption measurements were reproducible within a run, triplicate samples were analyzed. Figure 11 graphically represents these results. From the data analysis using Student's t distribution test with a 95% confidence

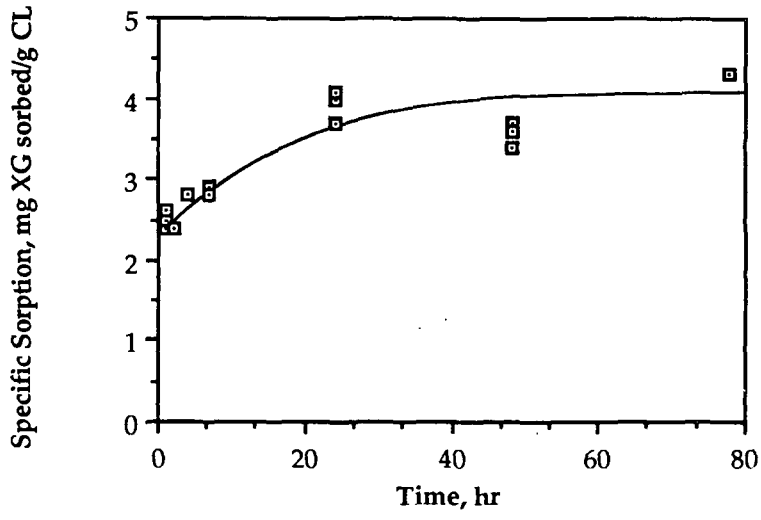


Figure 11. Time to sorption equilibrium and reproducibility within a sorption run. Sorption Experiment#2, 3827-16.

level, sorption measurements were shown to be reproducible within a run (Appendix V describes this form of statistical analysis).

Effect of Xyloglucan Concentration

The effect of xyloglucan concentration on sorption was examined. From the equilibrium adsorption isotherm, monolayer or multilayer adsorption can be determined. If monolayer adsorption occurs, the maximum specific sorption at equilibrium ($\Gamma_m$) can be calculated.

All sorption experiments indicated a rather rapid increase in specific sorption ($\Gamma$) with increasing equilibrium xyloglucan concentration ($C_e$). This was followed by a plateau region at higher $C_e$ values. Therefore, monolayer adsorption occurred. Data from these experiments is presented in Appendix IV and Figure 12 shows the

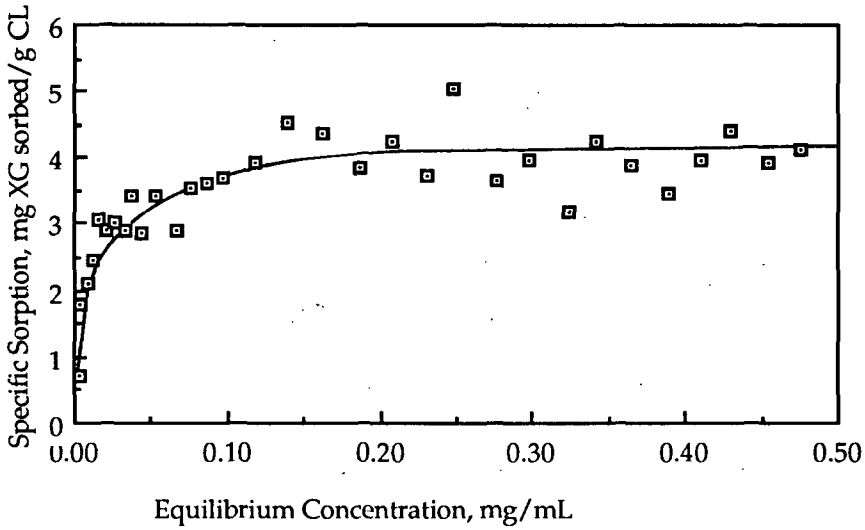


Figure 12. Adsorption isotherm illustrating effect of xyloglucan equilibrium concentration. Sorption Experiment#3C, 3827-39.

adsorption isotherm for one run.

Langmuir's adsorption isotherm theory was originally developed for gas molecules, but it has been adapted for polymer adsorption. [61] The Langmuir equation used for polymer adsorption is:

$$C^* = \frac{C^*_m K C_e}{1 + K C_e}$$

where $C^* = C_i - C_e$ = amount of polymer adsorbed

$C_i$ = initial polymer concentration

$C^*_m$ = maximum amount of polymer adsorbed

K = constant

This equation is inverted and rearranged to give a linearized form more appropriate for graphing:

$$\frac{C_e}{C^*} = \frac{1}{K C^*_m} + \frac{C_e}{C^*_m}$$

From a plot of $C_e/C^*$ vs. $C_e$ (Figure 13), $C^*_m$ can be determined from the slope. The maximum specific sorption ( $\Gamma_m$ ) is calculated from $C^*_m$, the cotton linters weight (CL), and the total sorption tube volume (TV) from the following equation:

$$\Gamma_m = \frac{C^*_m \times TV}{CL}$$

An average $\Gamma_m$ value of 3.9 mg xyloglucan sorbed per gram cotton linter was found.

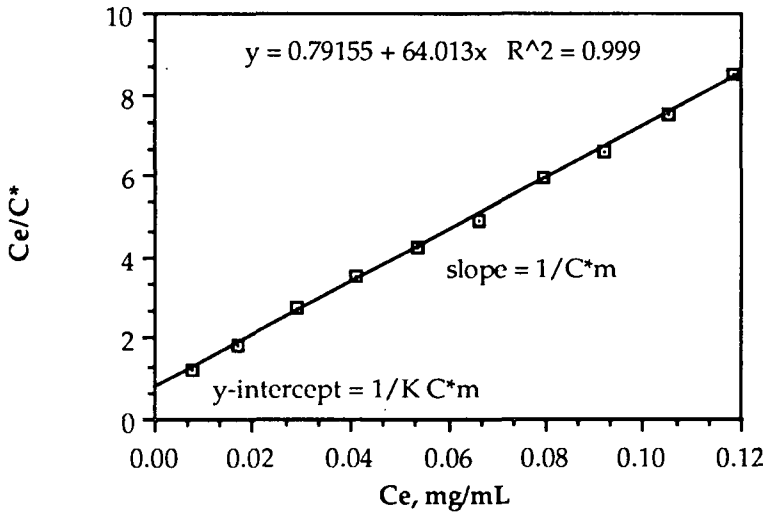


Figure 13. Langmuir plot of xyloglucan adsorbed onto cotton linters. Sorption Experiment#3E-1, 3827-51.

The Langmuir equation was used to analyze the data from the various experimental runs (Table 11). After the first four runs, it became apparent that the most reproducible results were obtained below a xyloglucan equilibrium concentration of 0.1 mg/mL. Above that concentration, a large amount of uncertainty was introduced by subtracting two large values ($C_i$ - $C_e$) to get a third ($C^*$), relatively very small number, e.g., for SE#3C, 3827-39-32, $C_i$ = 0.4928 mg/mL, $C_e$ = 0.4762 mg/mL, $C^*$ = 0.0166 mg/mL. Therefore, Sorption Experiments#3E-3G were run using xyloglucan equilibrium concentrations below or near 0.1 mg/mL. To eliminate possible error due to the use of a variable volume, automatic pipette use, the water and xyloglucan solution were weighed directly into the sorption tubes during these last three experiments.

Error Analyses of Sorption Experiment # 3

Due to reproducibility problems of $\Gamma_m$ in Sorption Experiment # 3, data scatter at higher xyloglucan concentrations, and possible distilled water contamination, this experiment was run seven times. Various forms of error analyses (see Appendix V) were performed. First, Student's t distribution test was run on the $\Gamma_m$ values calculated from SE#3E-3G. Not all of the $\Gamma_m$ values fell within the 95% confidence interval (3.9 ± 0.3), so further analyses were done.

Next, the confidence limits on the equilibrium concentration ($C_e$) and the calculated Langmuir term $C_e/C^*$ were examined. The confidence interval for $C_e$ was calculated using the Student's t test at 95% confidence limits. The confidence interval associated with $C_e/C^*$ is determined from an adaptation of standard error techniques (see Appendix V). Table 12 shows the results for this type of error analysis for SE#3G-1 and Figure 14 graphically illustrates the error by placing "error

Table 11. Calculated maximum specific sorption ( $\Gamma_m$ ) values for Sorption Experiment#3.

| Sorption Experiment | $\Gamma_m$ * mg XG/g CL |
|---|---|
| 3A | 4.6 |
| 3B | 5.5 |
| 3C | 3.8 |
| 3D | 4.5 |
| 3E-1 | 3.9 |
| 3E-2 | 3.8 |
| 3E-3 | 4.3 |
| 3E-4 | 4.3 |
| 3F-1 | 3.5 |
| 3F-2 | 3.4 |
| 3F-3 | 3.3 |
| 3F-4 | 3.3 |
| 3G-1 | 4.0 |
| 3G-2 | 4.1 |
| 3G-3 | 4.3 |
| 3G-4 | 4.5 |

* $\Gamma_m$ calculated using values of $C_e$ below approximately 0.1 mg/mL.

Table 12. Error associated with $C_e$ and $C_e/C^*$ for SE#3G-1.

| Sample | $C_e \pm \Delta C_e$ | $C_e/C^* \pm \Delta C_e/C^*$ |
|---|---|---|
| A | 0.0043 ± 0.0010 | 0.65 ± 0.25 |
| B | 0.0130 ± 0.0013 | 1.47 ± 0.37 |
| C | 0.0202 ± 0.0005 | 1.61 ± 0.11 |
| D | 0.0319 ± 0.0011 | 2.67 ± 0.34 |
| E | 0.0414 ± 0.0011 | 3.13 ± 0.35 |
| F | 0.0522 ± 0.0010 | 3.91 ± 0.39 |
| G | 0.0625 ± 0.0011 | 4.47 ± 0.43 |
| H | 0.0715 ± 0.0005 | 4.50 ± 0.18 |
| I | 0.0836 ± 0.0011 | 5.63 ± 0.53 |
| J | 0.0946 ± 0.0007 | 6.46 ± 0.49 |

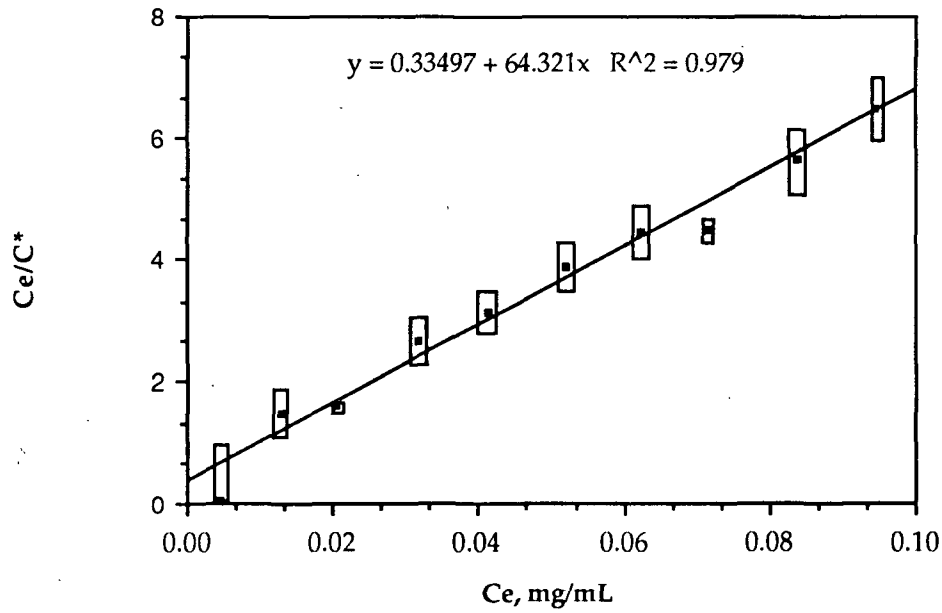


Figure 14. SE#3G-1 Langmuir plot with "error boxes".
Boxes represent confidence limits for $C_e$ and $C_e/C^*$.

boxes" around each point for SE#3G-1.

Confidence limits on $\Gamma_m$ as calculated from the error associated with the slope of the Langmuir plot of $C_e$ vs. $C_e/C^*$ can also be evaluated. Again, standard techniques for measuring error were adapted; Appendix V contains the details. Table 13 illustrates for various experiments the confidence limits for $\Gamma_m$ from this slope associated error.

Table 13. Error associated with the slope of the Langmuir plot for some sorption runs.

| Sorption Run | $\Gamma_m \pm \Delta\Gamma_m$ |
|---|---|
| SE#3F-1 | 3.52 ± 0.34 |
| SE#3F-2 | 3.44 ± 0.31 |
| SE#3F-3 | 3.29 ± 0.23 |
| SE#3G-1 | 4.08 ± 0.34 |
| SE#4-1 | 4.41 ± 0.35 |
| SE#4-2 | 4.54 ± 0.34 |
| SE#4-3 | 4.43 ± 0.53 |

The error analysis for the sorption runs demonstrates how large this error actually can be (i.e., $\Gamma_m$ ± 10% in some cases). Since this error is most likely caused by the subtraction of two large numbers ($C_i$ - $C_e$) from each other, the experimental techniques employed in this thesis only exacerbate this problem. Prevention of this would require a totally different experimental approach which was not within the scope of this thesis. This error explains some of the difficulties found in

reproducing $\Gamma_m$ in Sorption Experiment # 3.

Xyloglucan Sorption vs. Other Polymer Sorption Studies

The maximum specific sorption value can be used to compare xyloglucan with other polymers that have been sorbed onto cellulose fibers. Table 14 illustrates maximum equilibrium specific sorption values for other polymers. The cellulose substrate hydrodynamic specific surface area as measured by filtration resistance [48] is also included because this can affect the amount of sorption that takes place. Xyloglucan was found to have a specific sorption less than that of xylan, but similar to that of other hemicelluloses, cellulose derivatives, and other synthetic polymers on cellulose.

The approximate area each xyloglucan molecule occupies can be calculated from the maximum specific sorption, xyloglucan molecular weight, and the fiber specific surface area. With a fiber specific surface area of $1.06 \times 10^4$ $cm^2/g$, a value of $6.16 \times 10^{-12}$ $cm^2$/molecule (61,600 Angstroms squared per molecule) was found. Therefore, a value of 140 Angstroms was calculated as the radius of a circular area that the molecule would occupy. This is a reasonable value and is of the order of magnitude that would be predicted for the radius of gyration for a macromolecule of this molecular weight.

Effect Due to Molecular Weight

The sorption of different narrow molecular weight fractions of xyloglucan onto cellulose fibers was also examined. Three molecular weight fractions were isolated by size exclusion chromatography. From Langmuir's adsorption isotherm, a maximum specific sorption value for each molecular weight fraction was

Table 14. Maximum specific sorption of some hemicelluloses and other polymers onto cellulose fibers.

| Polymer | $\Gamma_m$, mg polymer sorbed/g cotton linters | Specific Surface Area of Cellulose Substrate, $m^2/g$ | Molecular Weight |
|---|---|---|---|
| Xyloglucan (this thesis) | 3.9 | 1.06 | $1.4 \times 10^6$ |
| Glucuronoarabinoxylan (GAX)[36] | 9.1 | 1.3 | 25,280 |
| GAX, 40% reduced | 12.0 | | |
| GAX, 60% reduced | 16.1 | | |
| Methyl Cellulose [62] | 3.6 | 1.42 | 15,000 |
| Hydroxy propyl cellulose | 3.9 | | $1 \times 10^6$ |
| Polygalactomannan | 2.0 | | * |
| Hydrolyzed poly(vinyl alcohol) | 4.1 | | $1.2 \times 10^6$ |
| Poly(vinyl methyl ether) | 5.1 | | 24,300 |

*Not a wood hemicellulose; intrinsic viscosity of 13.4 dL/g.

calculated (Appendix VI contains the details). Table 15 illustrates that xyloglucan molecular weight (over the molecular weight range of 0.2-1.9 x $10^6$) does not affect the maximum specific sorption of xyloglucan onto cellulose fibers.

Effect of Xyloglucan on Handsheet Properties

Since Laffend [34] and Walker [36] reported improved paper strength properties when the pulp was treated with hemicelluloses (glucomannan and glucuronoarabinoxylan respectively), a limited handsheet study was performed by The Institute of Paper Chemistry Pulp Lab staff using the xyloglucan isolated and

Table 15. Molecular weight fractions sorbed onto cotton linters and the calculated maximum specific sorption values.

| SEC fraction number | SEC MW | GPC/LALLS MW | Maximum Specific Sorption |
|---|---|---|---|
| 37-38-39 | $1.9 \times 10^6$ | $1.4 \times 10^6$ | 4.4 |
| 46-47-48 | $0.7 \times 10^6$ | $0.8 \times 10^6$ | 4.5 |
| 55-56-57 | $0.2 \times 10^6$ | $0.4 \times 10^6$ | 5.2 (4.4*) |

*The last three points of the graph for this sorption run do not fit the line that describes the rest of the graph; without these points, a value of 4.4 for $\Gamma_m$ is obtained.

characterized in this thesis. The details are presented in Appendix VII. Only minor improvements in the tensile index of the handsheets were found after xyloglucan addition.

Xyloglucan Sorption onto Cellulose

The significance of applying the results from *in vitro* studies to *in vivo* events is often unclear. Many factors must be evaluated and the most minor variable may be the key to the whole process. Therefore, caution is advised and only hypotheses and speculation are presented here.

The *in vitro* xyloglucan:cellulose relationship has been examined both in this thesis and by other researchers. Xyloglucan polymer and enzymatically degraded xyloglucan fragments have been adsorbed onto cellulose. A strong linkage between these two β-glucans (stronger in the native state than when sorbed *in vitro*) has been reported by previous researchers. [18,22] Careful evaluation of the xyloglucan:cellulose association was needed.

It is difficult to compare Valent and Albersheim's xyloglucan sorption study [18] to this thesis or to what takes place in the primary cell wall of plants. The use of non-physiological conditions (i.e., organic solvents) and xyloglucan fragments (rather than the intact polymer) could significantly alter the adsorption properties of this hemicellulose.

The failure of xyloglucan fragments to bind to cellulose in water was indicative, according to Valent and Albersheim [18], that the xyloglucan chain lifts off the cellulose surface and initiates xyloglucan chain creep. An alternative interpretation of this phenomenon can be presented using accepted polymer adsorption theory. For any solute, adsorption occurs when the free energy of the adsorbed molecule is less than that for the same molecule in the surrounding solution. For polymers, solubility increases as molecular weight decreases. Thus it is to be expected that xyloglucan fragments would have a high solubility in a good solvent (water) and a corresponding low potential for adsorption onto a surface. The converse (reduced solubility and ready sorption) would be expected for a high molecular weight xyloglucan. As discussed earlier, strong polymer adsorption (such as found in this thesis) does not disallow the inch-worm-like detachment and reattachment of a chain because the state of a given polymer segment is in dynamic equilibrium. Thus the lack of sorption from water of xyloglucan fragments is a matter of solubility and is not relevant to the postulated mechanism of xyloglucan chain creep.

Albersheim [17] hypothesized that the cellulose fibrils were covered by a xyloglucan monolayer in the primary cell wall. The plant cell wall model of Chambat, Barnoud, and Joseleau [12] indicated the possibility of xyloglucan to xyloglucan linkages or multilayers of xyloglucan. The *in vitro* xyloglucan

experiments of Hayashi [22] and this thesis found only monolayer sorption.

The amount of xyloglucan sorbed onto cellulose fibers is a function of many variables. The maximum specific sorption calculated in this thesis was 3.9 milligrams of xyloglucan sorbed per gram of cotton linters. Hayashi *et al.* [22] reported equilibrium at 5 micrograms of xyloglucan sorbed per 100 micrograms of pea cellulose (50 mg/g cellulose). The variation between these two values could be due to differences in specific surface area of the fibers (Hayashi does not report a value for surface area), temperature (Hayashi's experiments were run at 40°C), method of xyloglucan measurement (Hayashi used radioiodinated pea xyloglucan), and the xyloglucan itself (seed wall vs. cell wall xyloglucan). The native xyloglucan:cellulose complex, however, contains a much higher level of xyloglucan (14-fold higher according to Hayashi [22]) than this *in vitro* association. Hayashi hypothesized that this was because the xyloglucan binds not only to the microfibril surfaces in the primary cell wall, but also weaves into the amorphous regions of the microfibril. However, it seems unlikely that the amorphous regions of the microfibril could account for the presence of that much xyloglucan.

## CONCLUSIONS

The structure of xyloglucan isolated from *Tamarindus indica* in this thesis strongly resembled other tamarind xyloglucans reported in the literature. Due to the milder isolation procedure employed, the molecular weight of this polymer was much larger than previously found. No acetyl groups were found. The molecular weight and certain structural features (i.e., no fucose units) also differ from plant primary cell wall xyloglucan.

When sorbed onto well characterized cellulose fibers (cotton linters), this

xyloglucan exhibited equilibrium sorption within 24 hours. The equilibrium adsorption isotherm was defined. Monolayer sorption occurred. A maximum specific sorption value of 3.9 milligrams of xyloglucan adsorbed per gram of cotton linters was calculated. Maximum specific sorption was independent of molecular weight.

The amount of xyloglucan sorbed per gram of cellulose fiber was compared to that of other polymers. The xyloglucan from this study exhibited a maximum specific sorption less than xylan, a hemicellulose with ionized carboxyl groups, but similar to that of other hemicelluloses, cellulose derivatives, and synthetic polymers on cellulose.

Further work in this area is needed. A similar study to the present one, but using a carefully isolated primary cell wall xyloglucan, would clarify the effect of composition and branching on adsorption. Such knowledge could eventually help to explain what role the xyloglucan:cellulose association plays in the primary cell wall.

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And finally, "Yes, Norm, I must admit, it has been fun!"

## LITERATURE CITED


1. Aspinall, G. O. In Plant Carbohydrates II. Extracellular Carbohydrates. Tanner, W.; Loewus, F. A., eds. Springer-Verlag, New York, 1981.

2. Kooiman, P., Recl. Trav. Chim. Pays-Bas 80:849-865 (1961).

3. Srivastava, H. C.; Singh, P. P., Carbohydr. Res. 4:326-342 (1967).

4. White, E. V.; Rao, P. S., J. Am. Chem. Soc. 75:2617-2619 (1953).

5. Hsu, D. S.; Reeves, R. E., Carbohydr. Res. 5:202-209 (1967).

6. Kooiman, P., Phytochemistry 6:1665-1673 (1967).

7. Siddiqui, I. R.; Wood, P. J., Carbohydr. Res. 17:97-108 (1971).

8. Watanabe, T.; Shida, M.; Murayama, T.; Furuyama, Y.; Nakajima, T.; and Matsuda, K., Carbohyd. Res. 129:229-242 (1984).

9. Aspinall, G. O.; Fanous, H. K., Carbohydr. Polym. 4:193-214 (1984).

10. Simson, B. W.; Timell, T. E., Cellul. Chem. Technol. 12:51-62 (1978).

11. Hayashi, T.; Maclachlan, G., Plant Physiol. 75:596-604 (1984).

12. Chambat, G.; Barnoud, F.; Joseleau, J.-P., Plant Physiol. 74:687-693 (1984).

13. Aspinall, G. O.; Molloy, J. A.; Craig, J. W. T., Can. J. Biochem. 47:1063-1070 (1969).

14. Talmadge, K. W.; Keegstra, K.; Bauer, W. D.; Albersheim, P., Plant Physiol. 51:158-173 (1973).

15. Hayashi, T.; Kato, Y.; Matsuda, K., Plant Cell Physiol. 21(8):1405-1418 (1980).

16. Nealey, L. The isolation, characterization, and biological testing of xyloglucan from suspension cultured loblolly pine cell spent medium. Doctoral Dissertation, The Institute of Paper Chemistry, Appleton, Wisconsin, 1987.

17. Bauer, W. D.; Talmadge, K. W.; Keegstra, K.; Albersheim, P., Plant Physiol. 51:174-187 (1974).

18. Valent, B. S.; Albersheim, P., Plant Physiol. 54:105-108 (1974).

19. Keegstra, K.; Talmadge, K. W.; Bauer, W. D.; Albersheim, P., Plant Physiol. 51:188-196 (1973).

20. Lamport, D. T. A., Ann. Rev. Plant Physiol. 21:235-270 (1970).

21. Monro, J. A.; Penny, D.; Bailey, R. W., Phytochemistry 15:1193-1198 (1976).

22. Hayashi, T.; Marsden, M. P. F.; Delmer, D. P., Plant Physiol. 83:384-389 (1987).

23. Haigler, C. H.; Benziman, M., In Cellulose and Other Natural Polymer Systems. Brown, M., Jr., ed. Plenum, New York, 1982:23-43.

24. Labavitch, J. M.; Ray, P. M., Plant Physiol. 54:499-502 (1974).

25. Lorences, E. P.; Zarra, I., J. Exp. Bot. 38(191):960-967 (1987).

26. York, W. S.; Darvill, A. G.; Albersheim, P., Plant Physiol. 75:295-297 (1984).

27. Patat, F.; Killman, E.; Schliebener, C., Rubber Chem. Technol. 39:36-87 (1966).

28. Fleer, G. J.; Lyklema, J., In Adsorption from Solution at the Solid/Liquid Interface. Parfitt, G. D.; Rochester, C. H., eds. Academic Press, New York, 1983:153-220.

29. Silberberg, A., J. Phys. Chem. 66:1884-1907 (1962).

30. Kindler, W. A., Jr. Adsorption kinetics in the polyethyleneimine-cellulose fiber system. Doctoral Dissertation, The Institute of Paper Chemistry, Appleton, Wisconsin, 1971.

31. Cohen Stuart, M. A.; Scheutjens, J. M. H. M.; Fleer, G. J., J. Polym. Sci.; Polym. Phys. 18:559-573 (1980).

32. Most, D. S. The sorption of certain slash pine hemicellulose fractions by cellulose fibers. Doctoral Dissertation, The Institute of Paper Chemistry, Appleton, Wisconsin, 1957. Tappi 40(9):705-712 (1957).

33. Eriksson, E.; Samuelson, O.; Viale, A., Svensk Papperstidn. 66(10):403-406 (1963).

34. Laffend, K. The effect of acetyl content of glucomannan on its sorption onto cellulose and on its beaater additive properties. Doctoral Dissertation, The Institute of Paper Chemistry, Appleton, Wisconsin, 1967. Tappi 51(3):118-123 (1968).

35. Clayton, D. W.; Phelps, G. R., J. Polym. Sci.: Part C 11:197-220 (1965).

36. Walker, E. F. Effects of the uronic acid carboxyls on the sorption of 4-O-methylglucuronoarabinoxylans and their influence on the papermaking properties of cellulose fibers. Doctoral Dissertation, The Institute of Paper Chemistry, Appleton, Wisconsin, 1964. Tappi 48(5):298-303 (1965).

37. Dugal, H. S.; Swanson, J. W., Tappi 55(9):1362-1367 (1972).

38. Russo, V. A. Sorption studies of a modified locust bean gum on a bleached sulfite pulp. Doctoral Dissertation, The Institue of Paper Chemistry, Appleton, Wisconsin, 1959. Tappi 43(3):209-218 (1960).

39. Borchardt, L. G.; Piper, C. V., Tappi 53(10):257-260 (1970).

40. Tappi Standard Method T211 cm-80.

41. Ciucanu, I.; Kerek, F., Carbohydr. Res. 131:209-217 (1984).

42. Fengel, D..; Wegener, G.; Heizmann, A.; Przyklenk, M., Holzforsch. 31(3):65-71 (1977).

43. Kennedy, J. F.; Stevenson, D. L.; White, C. A.; Tolley, M. S.; Bradshaw, I. J., Br. Polym. J. 16(March):5-10 (1984).

44. Whistler, R. L.; Feather, M. S. In Methods in Carbohydrate Chemistry, Vol. 1. Whistler, R. L.; Wolfrom, M. L., eds. Academic Press, New York, 1962.

45. Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F., Anal. Chem. 28(3):350-356 (1956).

46. Gaillard, B. D. E.; Thompson, N. S.; Morak, A. J., Carbohydr. Res. 11:509-519 (1969).

47. McCarthy, W. R. An investigation of the mechanism of alkaline sizing with alkenyl succinic anhydride. Doctoral Dissertation, The Institute of Paper Chemistry, Appleton, Wisconsin, 1987.

48. Ingmanson, W. L.; Whitney, R. P., Tappi 37(11):523-528 (1954).

49. Stone, J.E.; Nickerson, L. F., Pulp Paper Mag. Can. 64(3):T155-T161 (1963).

50. Rao, P. S., In: Industrial Gums. Whistler, R. L., ed. Academic Press, New York, 1959:461-504.

51. Rydholm, S. A. Pulping Processes. Interscience, New York, 1965:1269.

52. Reis, D.; Vian, B.; Darzens, D.; Roland, J.-C., Planta 170:60-73 (1987).

53. Hakamori, S., J. Biochem. 55:205-208 (1964).

54. Björndahl, H.; Hellerqvist, C. G.; Lindberg, B.; Svensson, S., Agnew. Chem. Intern. Edit. 9(8):610-619 (1970).

55. Albersheim, P.; Nevins, D. J.; English, P. D.; Karr, A., Carbohydr. Res. 5:340-345 (1967).

56. Jansson, P.-E.; Kenne, L.; Liedgren, H.; Lindberg, B.; Lonngren, J., Chem. Commun. (Univ. of Stockholm) (8):1-75 (1976).

57. O'Neill, M. A.; Selvendran, R. R., Carbohydr. Res. 145:45-58 (1985).

58. Khan, N. A.; Mukherjee, B. D., Chem. Ind. (London) (1959):1413-1414.

59. McNeil, M.; Darvill, A. G.; Fry, S. C.; Albersheim, P., Ann. Rev. Biochem. 55:625-663 (1984).

60. York, W. S.; Oates, J. E.; van Halbeek, H.; Darvill, A. G.; Albersheim, P.; Tiller, P. R.; Dell, A., Carbohydr. Res. 173:113-132 (1988).

61. Shaw, D. J. Introduction to Colloid and Surface Chemistry. Butterworth, Boston, 1970.

62. Ishimaru, Y.; Lindstrom, T., J. Appl. Polym. Sci. 29:1675-1691 (1984).

63. Hill, C. G., Jr. An Introduction to Chemical Engineering Kinetics and Reactor Design. John Wiley, New York, 1977:594.

64. Instruction Manual for Model S/P 182 Electronic Analytical Balance. American Scientific Products, McGaw Park, Illinois.

# APPENDIX I

## XYLOGLUCAN ISOLATED FROM FRESH AND 20 YEARS OLD TAMARIND SEED POWDER

Questions arose concerning the possibility of xyloglucan degradation due to the age (twenty years) of the tamarind seed powder (TSP). Fresh tamarind seeds were, therefore, obtained from Dr. H. S. Dugal; the xyloglucan was isolated and then analyzed. The analytical results for the xyloglucan isolated from Dugal's tamarind seeds (TS) and the 20 years old TSP are shown in Table 16. No discernible differences between the isolated xyloglucans were apparent.

Table 16. Chemical characterization of xyloglucan isolated from fresh and old TSP.

| | Dugal's TS | | 20 years old TSP | |
|---|---|---|---|---|
| | Crude XG 3636-66-1 | Purified XG 3636-66-2 | Crude XG 3636-67-1 | Crude XG 3636-67-2 |
| Nitrogen, % | 0.28 | <0.1 | <0.1 | <0.1 |
| Sugar Analysis | | | | |
| Araban, % | 1.5 | 1.4 | 1.4 | 1.4 |
| Xylan, % | 29.8 | 30.6 | 28.4 | 28.8 |
| Mannan, % | 1.0 | 0.8 | 1.1 | 1.5 |
| Galactan, % | 14.7 | 15.2 | 14.6 | 15.4 |
| Glucan, % | 50.1 | 51.9 | 50.3 | 49.8 |
| Total Carbohydrate, % | 97.1 | 99.9 | 95.8 | 96.9 |
| Ash, % | 0.71 | 0.58 | 0.19 | 0.22 |
| Functional Groups | NGL | NGL | NGL | NGL |

# APPENDIX II

## XYLOGLUCAN TOTAL ION CHROMATOGRAM AND MASS SPECTRA

The total ion chromatogram (Figure 15) and mass spectra (Figures 16-22) of each peak for the partially methylated alditol acetates of xyloglucan are shown below and on the following pages. The peaks are labeled with the retention times (RT) from Figure 7. A discussion on interpreting mass spectra, e.g., Figure 21, the peak at 31.03 min RT, follows Figure 22. Resolution of the gas liquid chromatography and gas liquid chromatography/mass spectroscopy data for xyloglucan may be seen in Table 8.

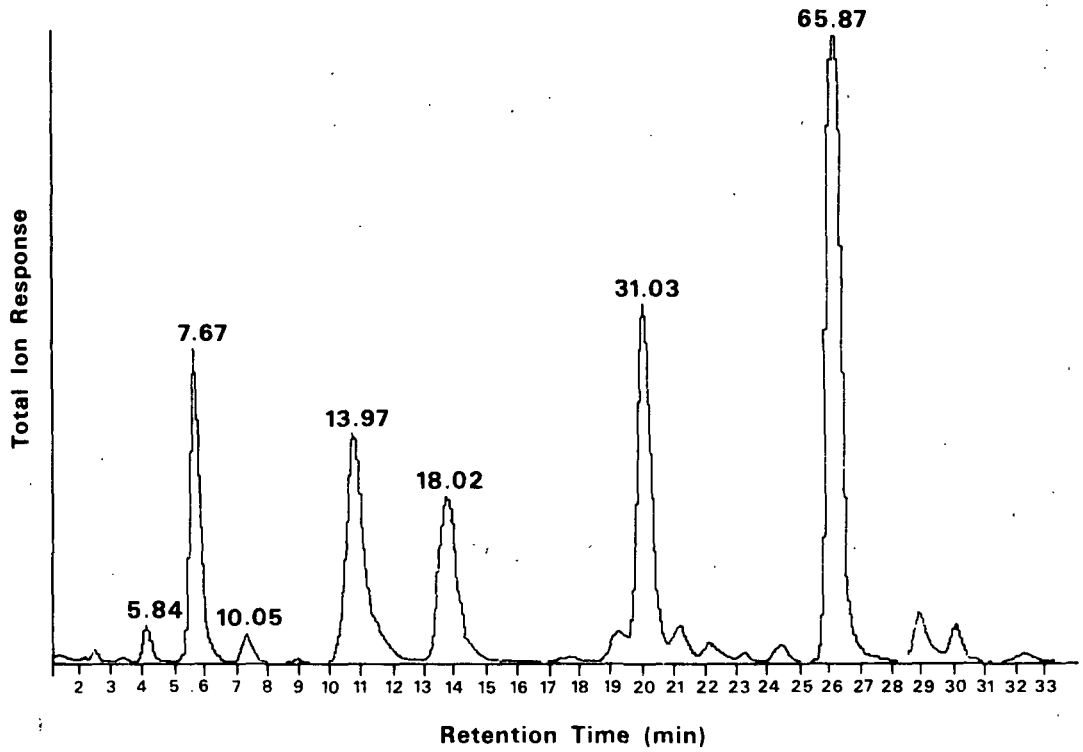



Figure 15. Total ion chromatogram of the partially methylated alditol acetates of xyloglucan.

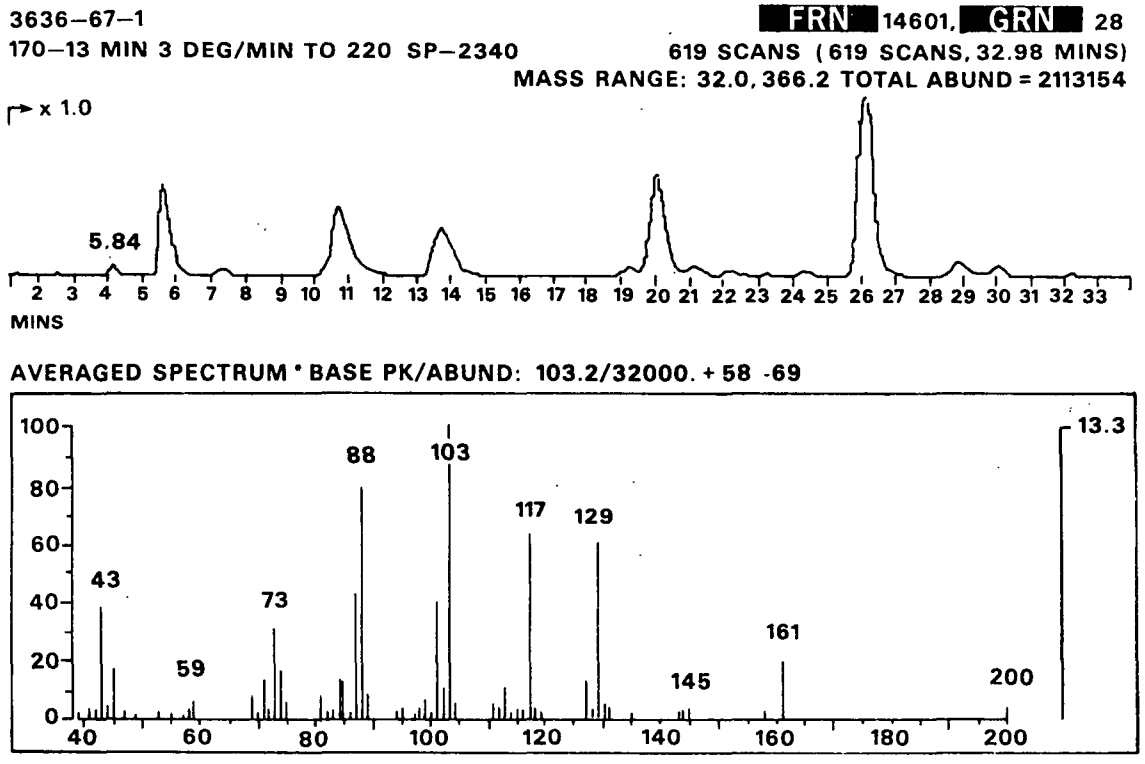



Figure 16. Mass spectra for the peak at 5.84 min RT.

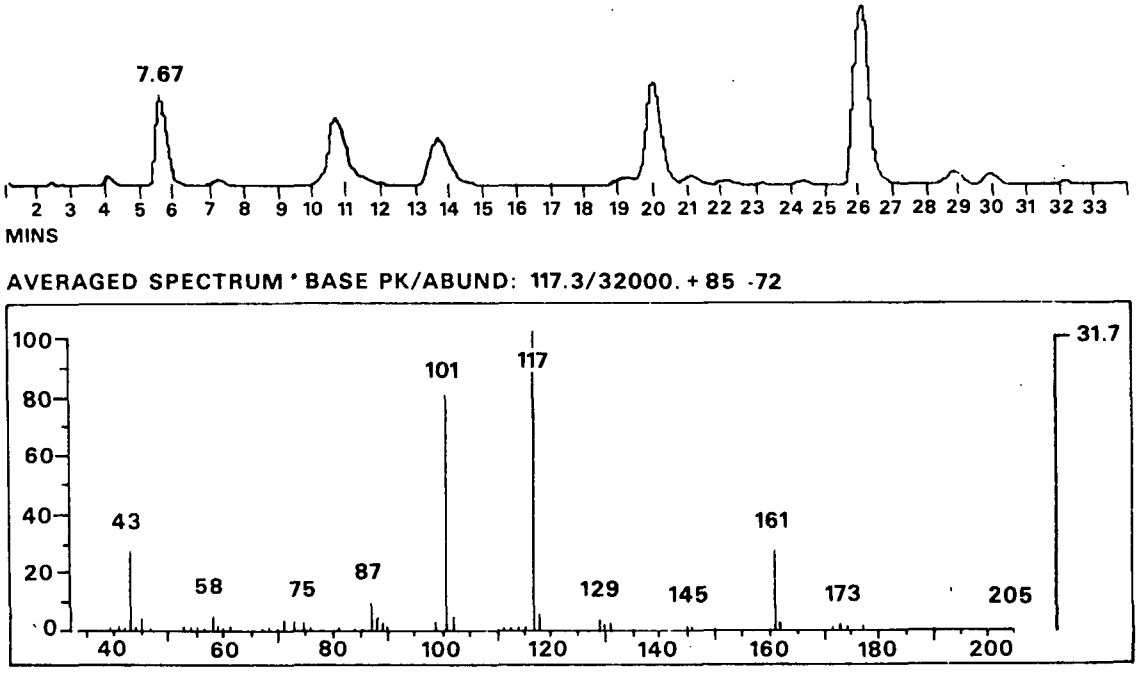



Figure 17. Mass spectra for the peak at 7.67 min RT.

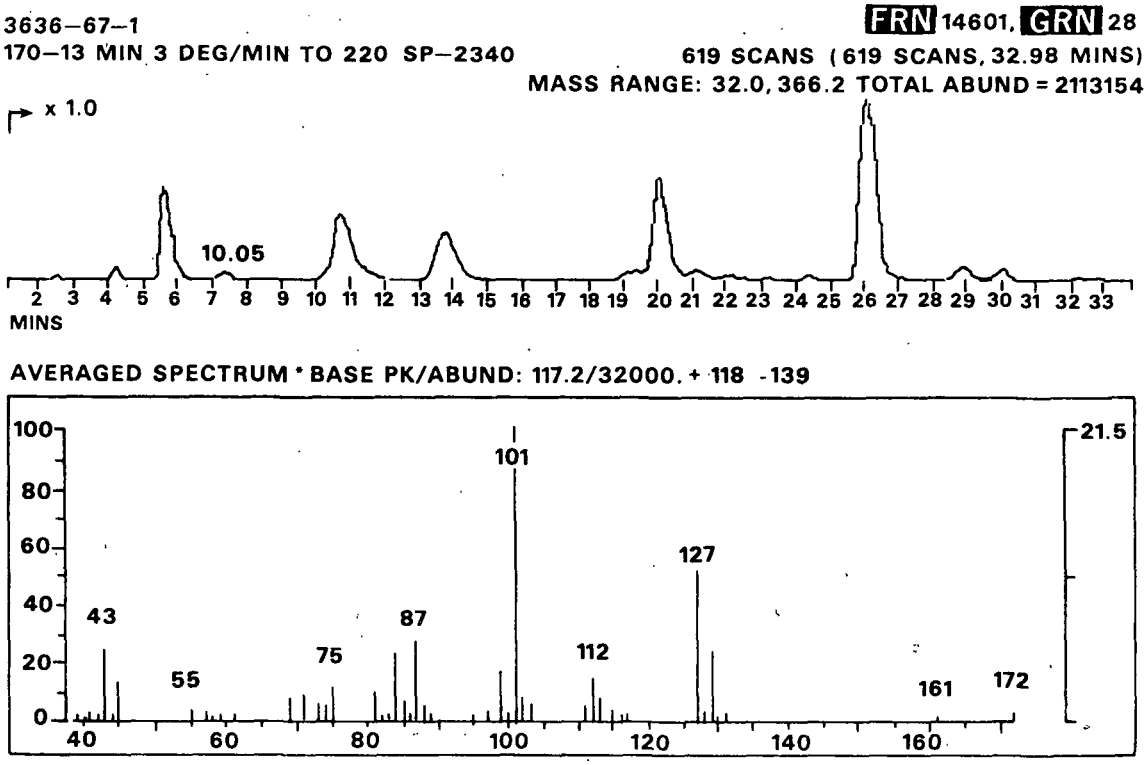



Figure 18. Mass spectra for the peak at 10.05 min RT.

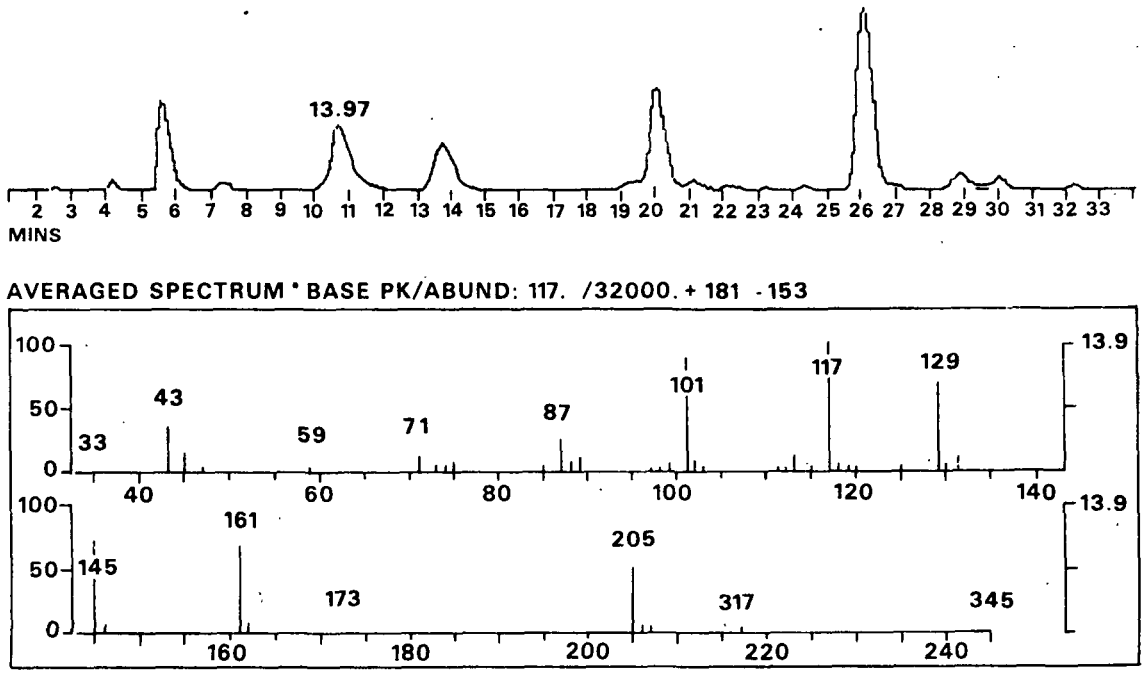



Figure 19. Mass spectra for the peak at 13.97 min RT.

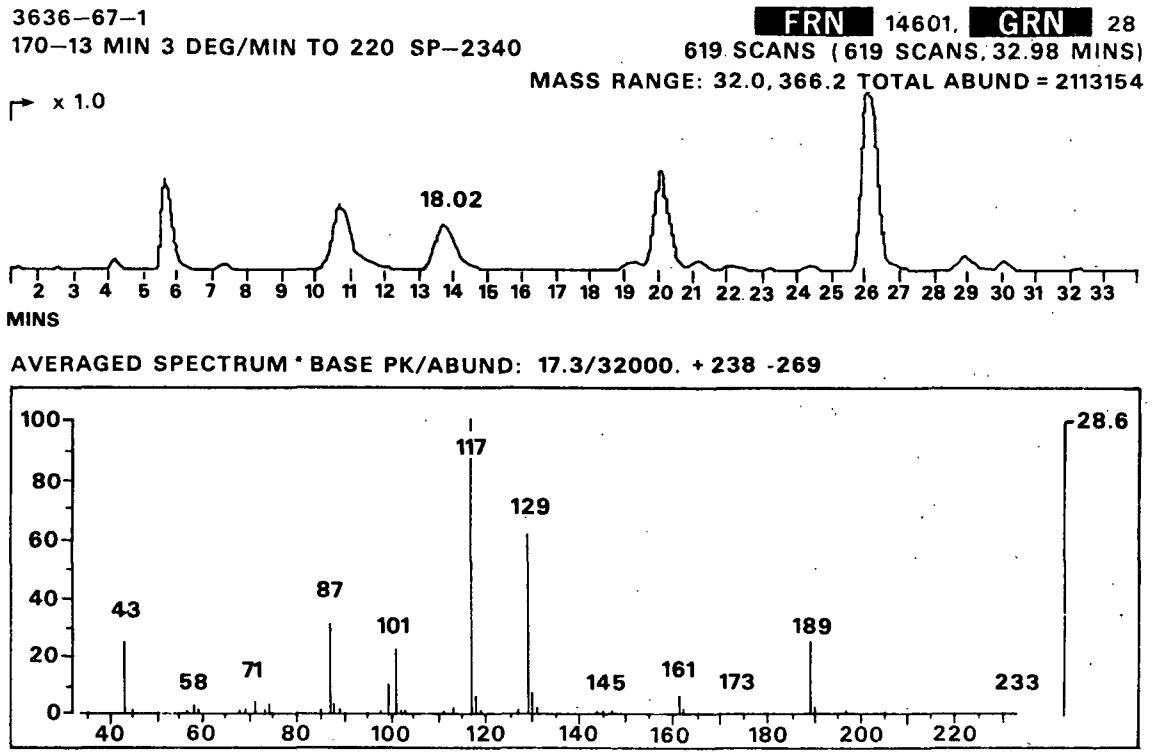



Figure 20. Mass spectra for the peak at 18.02 min RT.

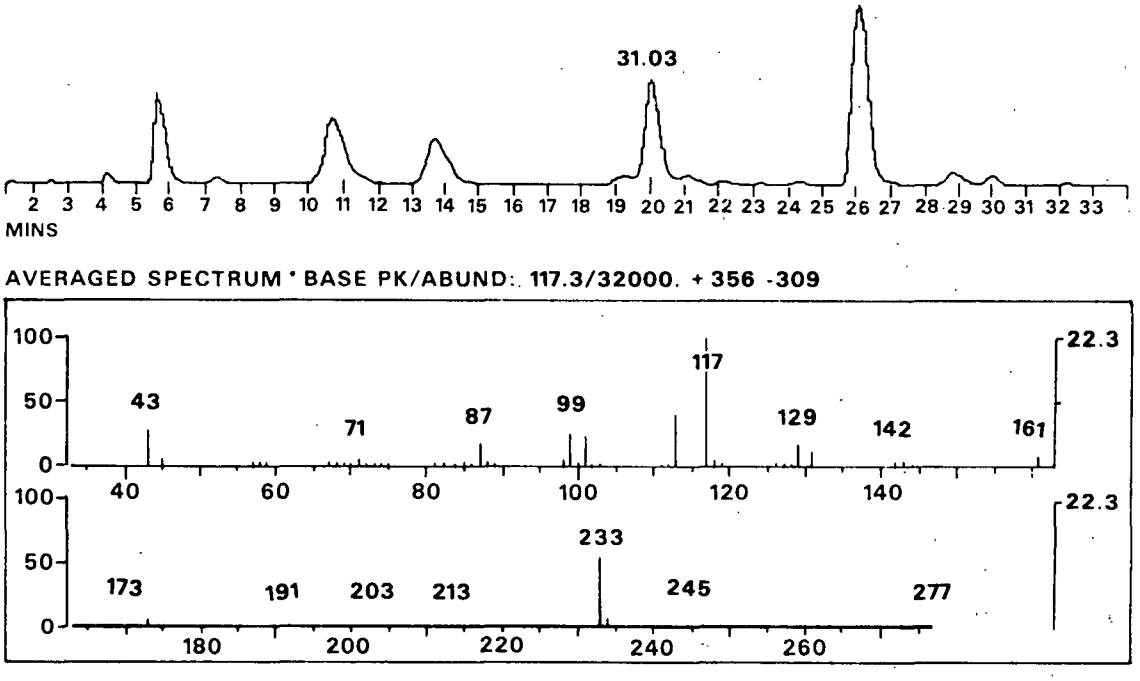



Figure 21. Mass spectra for the peak at 31.03 min RT.

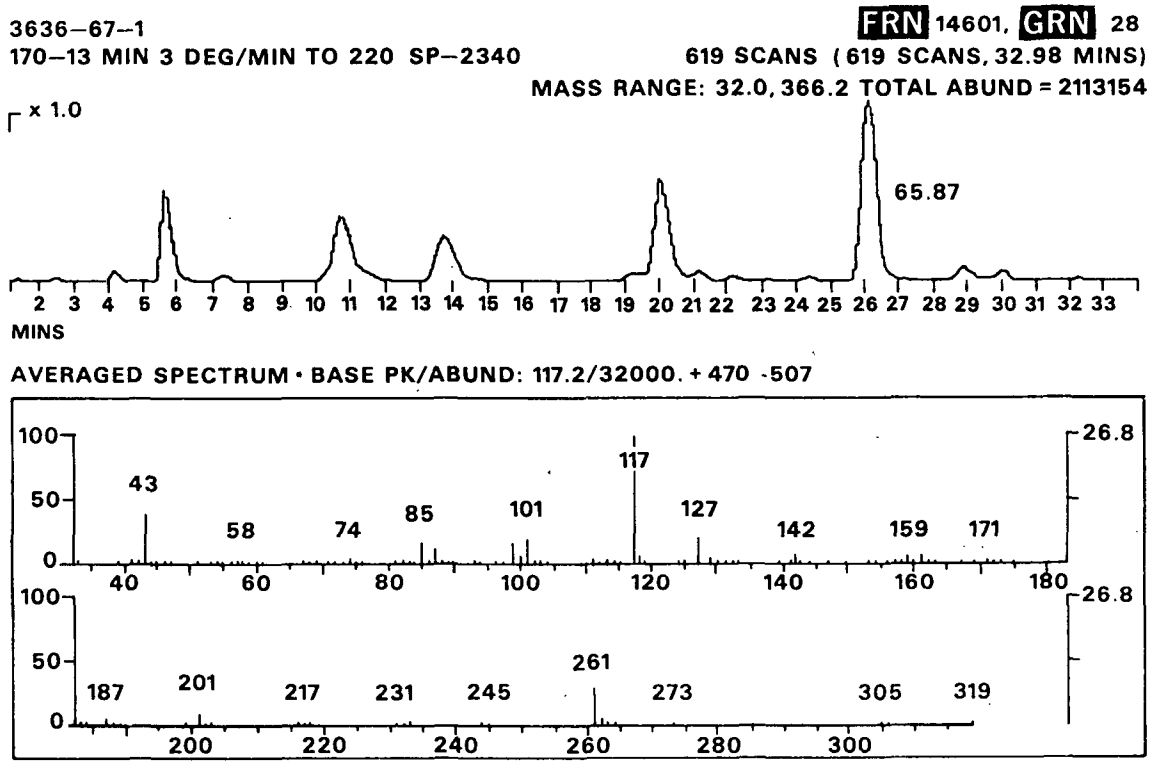



Figure 22. Mass spectra for the peak at 65.87 min RT.

Interpretation of the mass spectra of methylated alditol acetates was reviewed by Björndahl *et al.* [54] Certain fragmentation patterns are observed for these molecules. As an example, the mass spectrum of the peak at 31.03 min RT (236 Glu) contains major fragments at *m/e* (mass divided by charge) values of 43, 117, and 233; smaller fragments were found at *m/e* 45, 99, 101, 113, 129, 161, and 277. The base peak is generally *m/e* 43 ($CH_3C{=}O$, see Figure 23). Fission between two adjacent methoxylated carbons is preferred over fission between a methoxylated and an acetoxylated carbons or between two acetoxylated carbons. Therefore, the fragments at *m/e* 117, 161, and 233 are explained. Elimination of acetic acid (*m/e* 60), ketene (*m/e* 42), methanol 32, or formaldehyde (*m/e* 30) from these fragments also occurs.

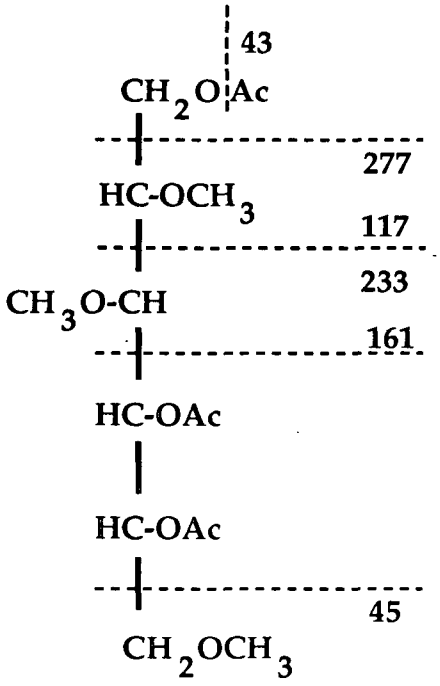



Figure 23. Possible fragmentation patterns for 236 Glu. [54]

# APPENDIX III

## SIZE EXCLUSION CHROMATOGRAPHY OF DEXTRAN MOLECULAR WEIGHT MARKERS AND XYLOGLUCAN

The data for the size exclusion chromatogram of xyloglucan (Figure 9) is shown below in Table 17. Figures 24-28 contain the size exclusion chromatograms for the dextran molecular weight markers.

Table 17. Data from the SEC of xyloglucan on Sepharose CL-4B, 0.1N NaOH eluant at 60 mL/h, 5 mL/fraction collected.

| Fraction Number | Xyloglucan Concentration, mg/mL |
|---|---|
| 32 | 0.0079 |
| 35 | 0.0672 |
| 38 | 0.1288 |
| 41 | 0.1355 |
| 44 | 0.1210 |
| 47 | 0.0926 |
| 50 | 0.0716 |
| 53 | 0.0524 |
| 56 | 0.0359 |
| 59 | 0.0238 |
| 62 | 0.0137 |
| 65 | 0.0099 |
| 68 | 0.0059 |
| 71 | 0.0036 |
| 74 | 0.0022 |
| 77 | 0.0016 |
| 80 | 0.0017 |

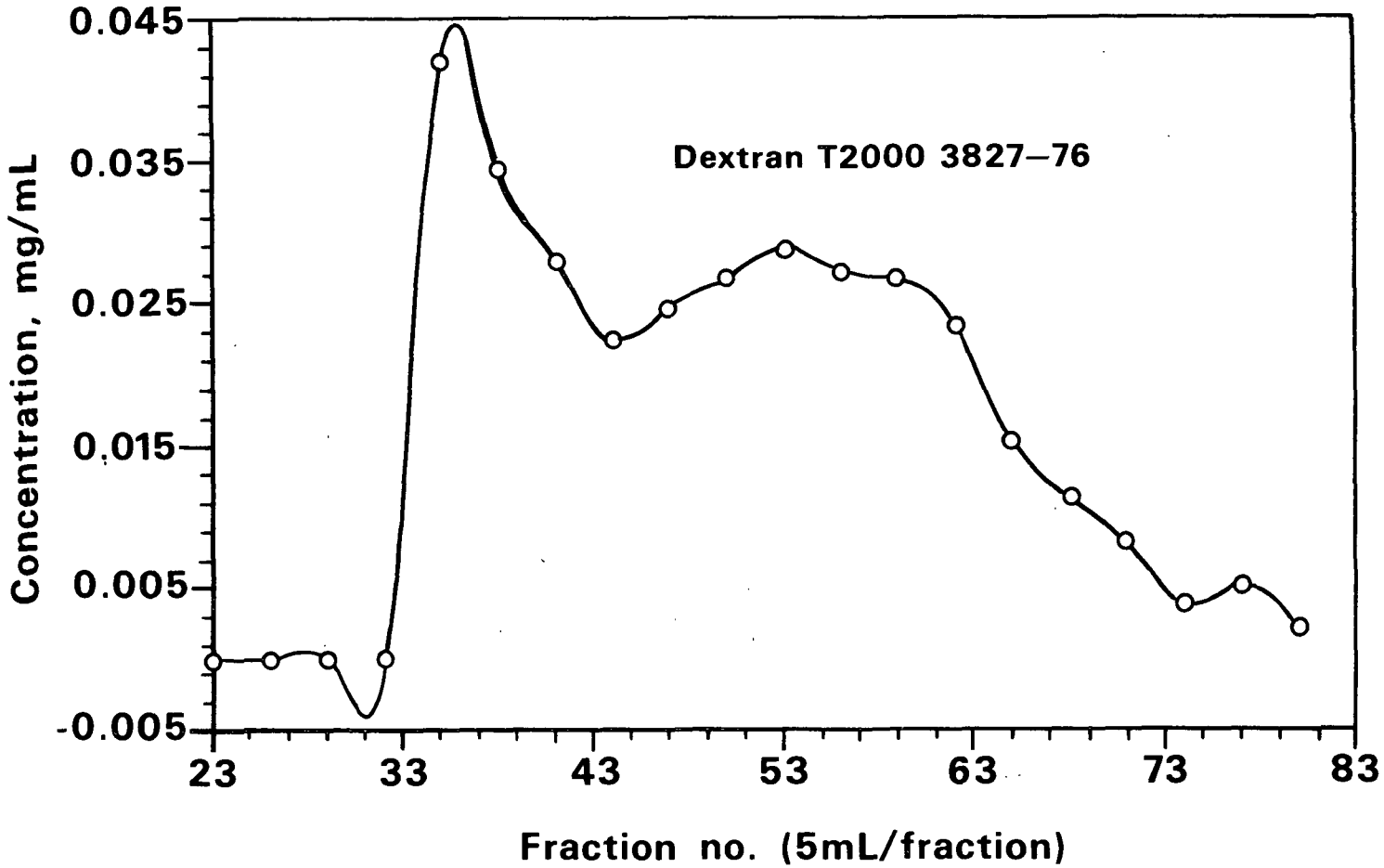



Figure 24. Size exclusion chromatogram of Dextran T2000.

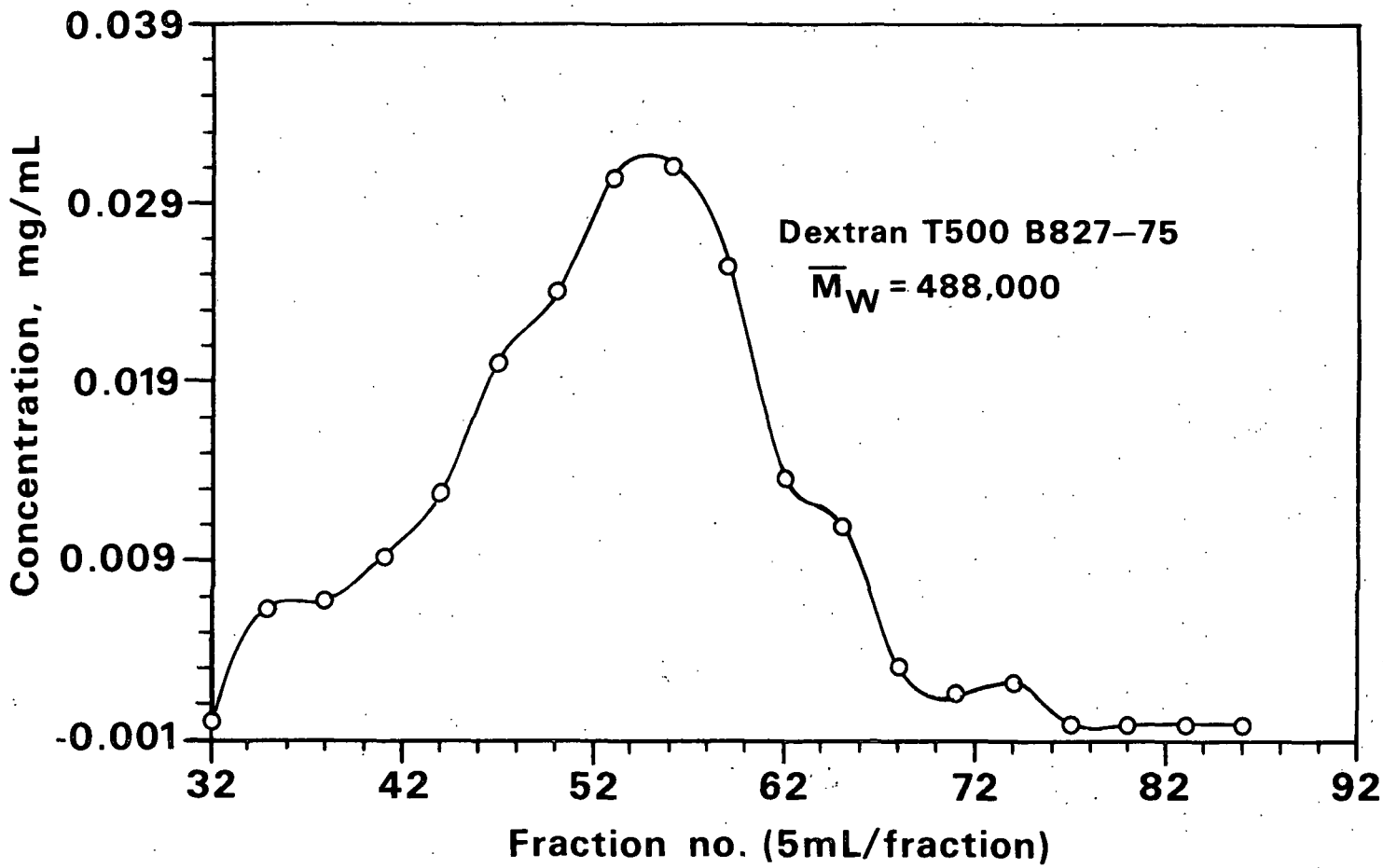



Figure 25. Size exclusion chromatogram of Dextran T500.

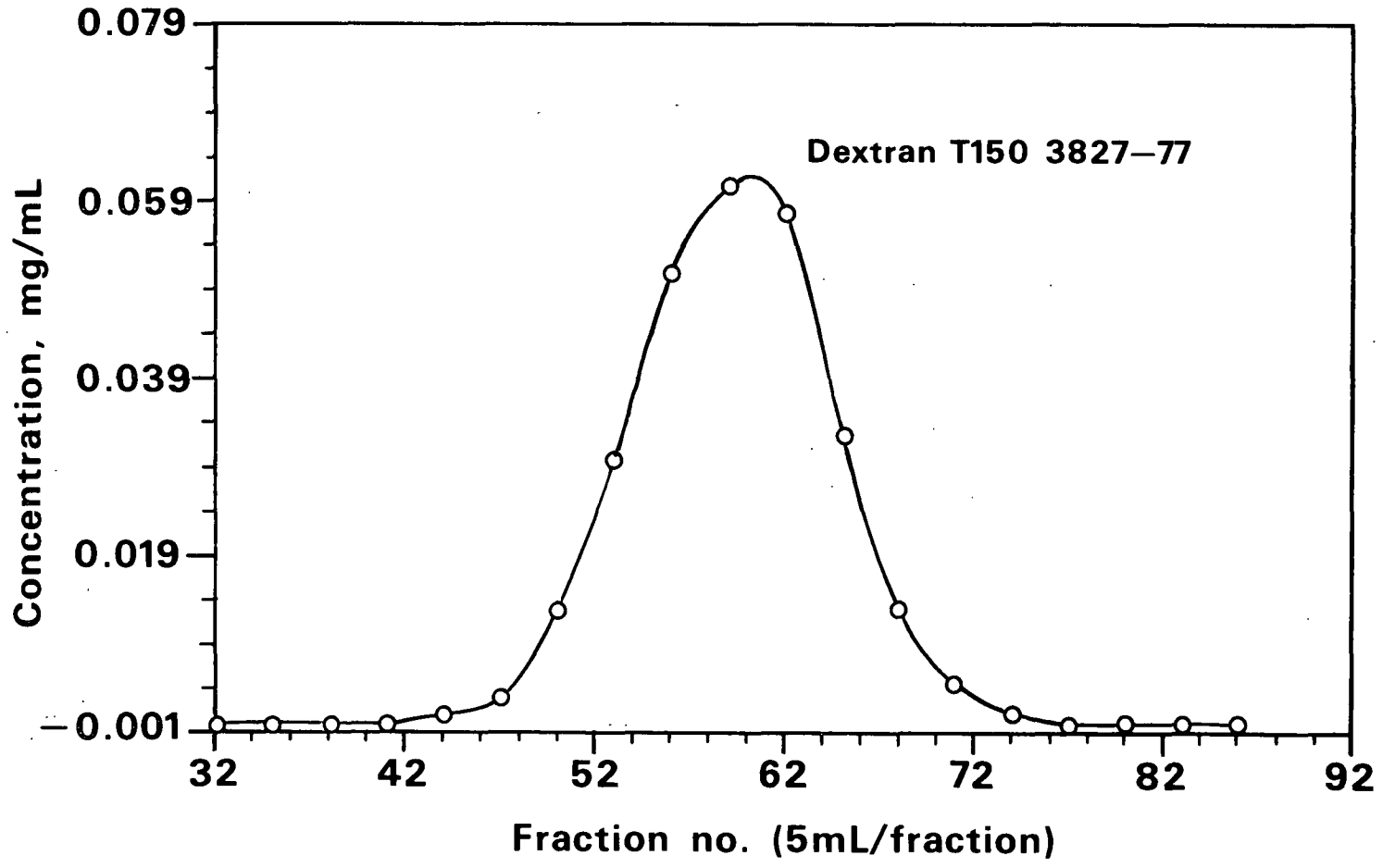



Figure 26. Size exclusion chromatogram of Dextran T150.

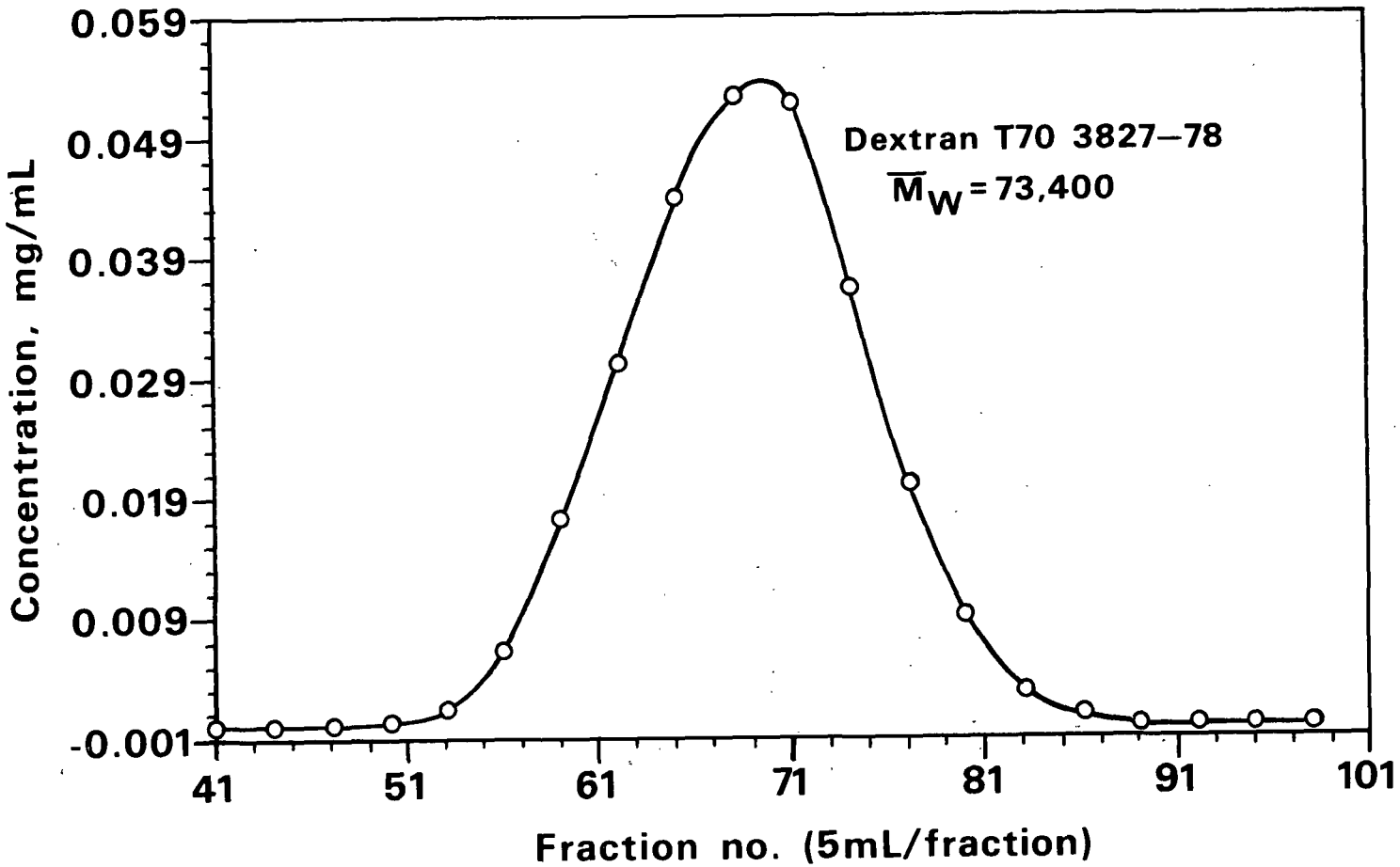



Figure 27. Size exclusion chromatogram of Dextran T70.

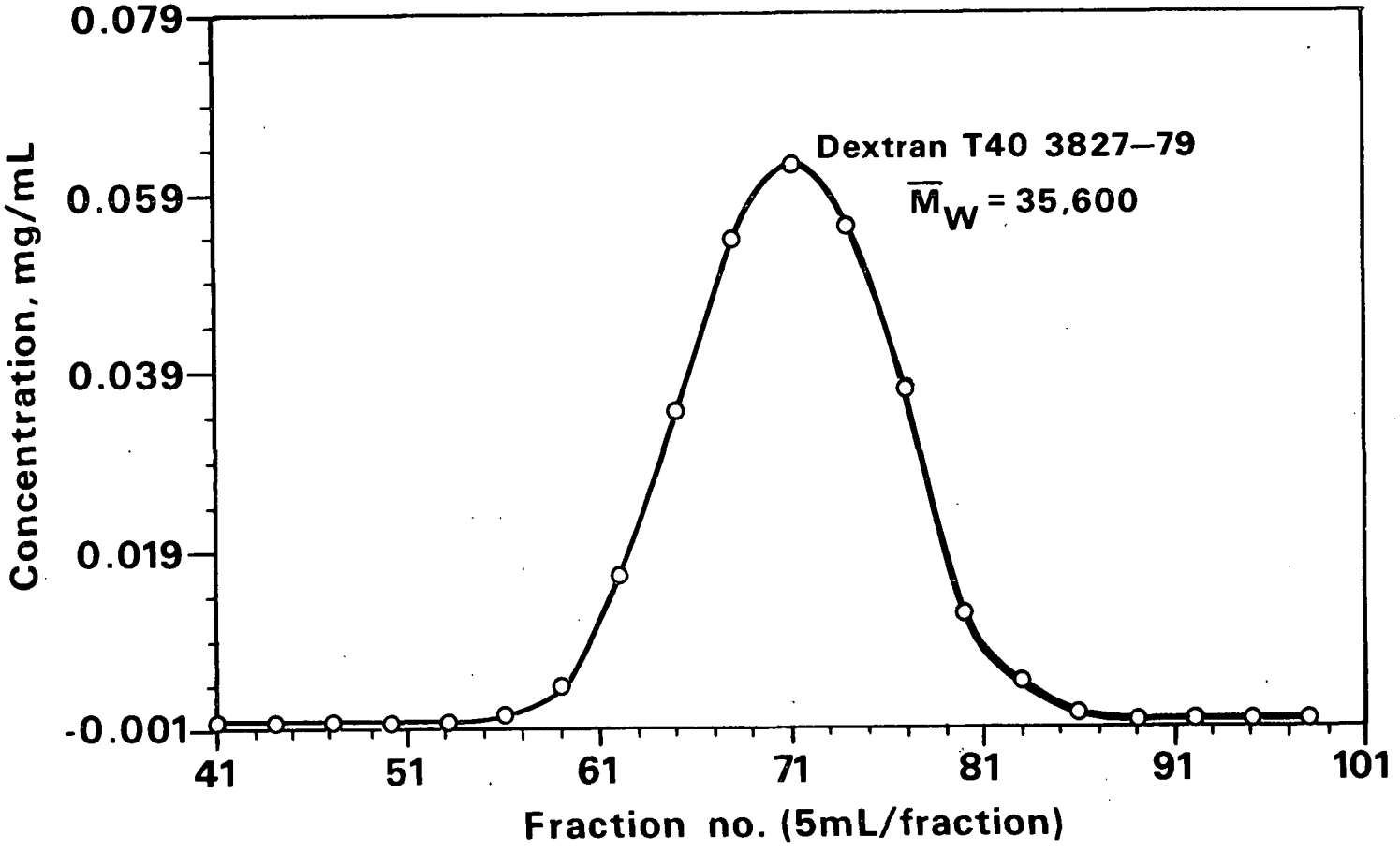



Figure 28. Size exclusion chromatogram of Dextran T40.

# APPENDIX IV

## DATA FROM SORPTION EXPERIMENTS

SE#1, 3827-12
Initial Xyloglucan Concentration : 0.1279 mg/mL

| Sample, Time, hr | Concentration, mg/mL | Specific Sorption, mg XG sorbed/g CL |
|---|---|---|
| 0.17 | 0.1225 | 1.35 |
| 0.50 | 0.1220 | 1.47 |
| 1.00 | 0.1181 | 2.45 |
| 2.08 | 0.1152 | 3.18 |
| 3.92 | 0.1160 | 2.98 |
| 7.00 | 0.1148 | 3.29 |
| 12.33 | 0.1142 | 3.43 |
| 24.55 | 0.1122 | 3.93 |
| 32.55 | 0.1142 | 3.43 |
| 48.22 | 0.1157 | 3.06 |
| 72.92 | 0.1120 | 3.99 |

SE#2, 3827-16
Initial Xyloglucan Concentration : 0.0832 mg/mL

| Sample, Time, hr | Concentration, mg/mL | Specific Sorption, mg XG sorbed/g CL |
|---|---|---|
| 0.5 | 0.0718 | 2.85 |
| 1.0 - A | 0.0734 | 2.44 |
| B | 0.0730 | 2.55 |
| C | 0.0731 | 2.53 |
| 2.28 | 0.0735 | 2.42 |
| 3.92 | 0.0721 | 2.78 |
| 7.05 - A | 0.0716 | 2.89 |
| B | 0.0715 | 2.93 |
| C | 0.0720 | 2.81 |
| 24.00 - A | 0.0671 | 4.03 |
| B | 0.0683 | 3.73 |
| C | 0.0668 | 4.10 |
| 48.30 - A | 0.0694 | 3.45 |
| B | 0.0685 | 3.67 |
| C | 0.0687 | 3.63 |
| 77.63 - A | 0.0661 | 4.26 |
| B | 0.0661 | 4.27 |

SE#2B, 3827-20
Initial Xyloglucan Concentration : 0.0817 mg/mL

| Sample, Time, hr | Concentration, mg/mL | Specific Sorption, mg XG sorbed/g CL |
|---|---|---|
| 0.50 | 0.0728 | 2.23 |
| 1.08 | 0.0726 | 2.27 |
| 2.00 | 0.0722 | 2.38 |
| 4.83 | 0.0716 | 2.51 |
| 7.05 | 0.0691 | 3.14 |
| 16.55 | 0.0672 | 3.63 |
| 21.00 | 0.0683 | 3.35 |
| 24.00 | 0.0666 | 3.74 |
| 26.50 | 0.0665 | 3.78 |
| 31.00 | 0.0671 | 3.65 |
| 40.45 | 0.0659 | 3.95 |
| 44.18 | 0.0679 | 3.45 |
| 46.90 | 0.0672 | 3.63 |
| 50.70 | 0.0664 | 3.83 |
| 56.30 | 0.0659 | 3.95 |
| 66.42 | 0.0653 | 4.11 |

SE#3A, 3827-24

| Sample | $C_e$, mg/mL | $C_e/C^*$ | Specific Sorption* | |
|---|---|---|---|---|
| 1 | 0.0015 | 0.34 | 1.10 | |
| 2 | 0.0048 | 0.69 | 1.75 | |
| 3 | 0.0123 | 1.09 | 2.80 | |
| 4 | 0.0223 | 1.70 | 3.24 | |
| 5 | 0.0348 | 2.81 | 3.11 | $\Gamma_m$ = 4.6 mg/g |
| 6 | 0.0438 | 2.88 | 3.76 | |
| 7 | 0.0541 | 3.26 | 4.15 | |
| 8 | 0.0652 | 3.77 | 4.33 | K = 125.0 mL/mg |
| 9 | 0.0772 | 4.51 | 4.30 | |
| 10 | 0.1013 | 6.10 | 4.16 | |
| 11 | 0.1247 | 7.42 | 4.19 | |
| 12 | 0.1457 | 7.51 | 4.83 | |
| 13 | 0.1728 | 10.94 | 3.93 | |
| 14 | 0.1935 | 10.35 | 4.65 | |
| 15 | 0.2188 | 12.87 | 4.23 | |
| 16 | 0.2394 | 11.97 | 4.96 | |
| 17 | 0.2622 | 12.61 | 5.19 | |

* Specific Sorption = mg xyloglucan sorbed per g cotton linters

SE#3B, 3827-29

| Sample | $C_e$, mg/mL | $C_e/C^*$ | Specific Sorption | |
|---|---|---|---|---|
| 1 | 0.0027 | 0.79 | 0.85 | |
| 2 | 0.0039 | 0.47 | 2.06 | |
| 3 | 0.0083 | 0.83 | 2.47 | |
| 4 | 0.0117 | 0.92 | 3.17 | |
| 5 | 0.0181 | 1.46 | 3.09 | $\Gamma_m$ = 5.5 mg/g |
| 6 | 0.0215 | 1.42 | 3.78 | |
| 7 | 0.028 | 1.90 | 3.65 | |
| 8 | 0.0333 | 2.15 | 3.88 | K = 100.0 mL/mg |
| 9 | 0.0359 | 1.89 | 4.73 | |
| 10 | 0.0425 | 2.30 | 4.61 | |
| 11 | 0.0551 | 3.06 | 4.47 | |
| 12 | 0.0667 | 3.59 | 4.68 | |
| 13 | 0.0784 | 4.10 | 4.74 | |
| 14 | 0.0887 | 4.22 | 5.25 | |
| 15 | 0.1023 | 5.22 | 4.90 | |
| 16 | 0.1243 | 5.95 | 4.61 | |
| 17 | 0.1478 | 6.45 | 5.74 | |
| 18 | 0.1695 | 8.11 | 5.23 | |
| 19 | 0.1957 | 8.26 | 5.95 | |
| 20 | 0.2186 | 11.27 | 4.83 | |
| 21 | 0.2412 | 8.93 | 6.70 | |
| 22 | 0.2635 | 11.92 | 5.52 | |
| 23 | 0.2912 | 11.33 | 6.42 | |
| 24 | 0.3096 | 13.12 | 5.87 | |
| 25 | 0.3385 | 12.44 | 6.79 | |
| 26 | 0.3572 | 15.14 | 5.89 | |
| 27 | 0.3910 | 16.64 | 5.81 | |
| 28 | 0.4039 | 16.49 | 6.10 | |
| 29 | 0.4463 | 26.41 | 4.20 | |
| 30 | 0.4642 | 39.01 | 2.97 | |
| 32 | 0.4895 | 21.76 | 5.60 | |
| 33 | 0.5143 | 54.71 | 2.34 | |
| 34 | 0.5301 | 17.32 | 7.66 | |
| 35 | 0.5624 | 24.78 | 5.68 | |
| 36 | 0.5733 | 15.84 | 9.05 | |

SE#3C, 3827-39

| Sample | $C_e$, mg/mL | Ce/C* | Specific Sorption* | |
|---|---|---|---|---|
| 1 | 0.0028 | 0.97 | 0.71 | |
| 2 | 0.0041 | 0.58 | 1.77 | |
| 3 | 0.0083 | 0.98 | 2.10 | |
| 4 | 0.0126 | 1.29 | 2.45 | |
| 5 | 0.0158 | 1.30 | 3.06 | $\Gamma_m$ = 3.8 mg/g |
| 6 | 0.0220 | 1.90 | 2.90 | |
| 7 | 0.0272 | 2.27 | 3.00 | |
| 8 | 0.0333 | 2.90 | 2.90 | K = 126.1 mL/mg |
| 9 | 0.0367 | 2.68 | 3.40 | |
| 10 | 0.0445 | 3.87 | 2.89 | |
| 11 | 0.0535 | 3.91 | 3.40 | |
| 12 | 0.0668 | 5.76 | 2.89 | |
| 13 | 0.0755 | 5.35 | 3.54 | |
| 14 | 0.0864 | 6.00 | 3.62 | |
| 15 | 0.0972 | 6.57 | 3.71 | |
| 16 | 0.1187 | 7.56 | 3.93 | |
| 17 | 0.1388 | 7.71 | 4.53 | |
| 18 | 0.1616 | 9.18 | 4.38 | |
| 19 | 0.1863 | 12.18 | 3.84 | |
| 20 | 0.2069 | 12.10 | 4.26 | |
| 21 | 0.2315 | 15.54 | 3.72 | |
| 22 | 0.2484 | 12.18 | 5.05 | |
| 23 | 0.2767 | 19.08 | 3.65 | |
| 24 | 0.2977 | 18.72 | 3.98 | |
| 25 | 0.3233 | 25.46 | 3.16 | |
| 26 | 0.3413 | 19.96 | 4.25 | |
| 27 | 0.2653 | 23.57 | 3.88 | |
| 28 | 0.3894 | 28.22 | 3.44 | |
| 29 | 0.4098 | 25.94 | 3.96 | |
| 31 | 0.4546 | 28.77 | 3.95 | |
| 32 | 0.4762 | 28.69 | 4.17 | |

SE#3D, 3827-47

| Sample | $C_e$, mg/mL | $C_e/C^*$ | Specific Sorption | |
|---|---|---|---|---|
| 1 | 0.0293 | 2.23 | 3.30 | |
| 2 | 0.0334 | 2.42 | 3.46 | |
| 3 | 0.0409 | 2.60 | 3.92 | |
| 4 | 0.0490 | 2.87 | 4.25 | |
| 5 | 0.0606 | 4.07 | 3.72 | $\Gamma_m$ = 4.5 mg/g |
| 6 | 0.0685 | 4.18 | 4.08 | |
| 7 | 0.0793 | 5.27 | 3.75 | |
| 8 | 0.0966 | 5.81 | 4.16 | K = 109.6 mL/mg |
| 9 | 0.1126 | 5.78 | 4.84 | |
| 10 | 0.1315 | 6.76 | 4.84 | |
| 11 | 0.1489 | 7.11 | 5.19 | |
| 12 | 0.1673 | 7.82 | 5.37 | |
| 13 | 0.1810 | 6.81 | 6.67 | |
| 14 | 0.1999 | 7.53 | 6.64 | |
| 15 | 0.2201 | 8.73 | 6.32 | |
| 16 | 0.2461 | 13.61 | 4.48 | |
| 17 | 0.2564 | 9.62 | 6.59 | |
| 18 | 0.2741 | 9.85 | 6.92 | |
| 19 | 0.2950 | 11.44 | 6.39 | |
| 20 | 0.3169 | 13.92 | 5.67 | |
| 21 | 0.3328 | 12.93 | 6.39 | |
| 22 | 0.3537 | 14.92 | 5.93 | |
| 23 | 0.3696 | 13.86 | 6.72 | |

SE#3E, 3827-51

| Sample | $C_e$, mg/mL | $C_e/C^*$ | Specific Sorption | |
|---|---|---|---|---|
| 1A | 0.0074 | 1.26 | 1.46 | |
| 1B | 0.0171 | 1.82 | 2.37 | |
| 1C | 0.0291 | 2.72 | 2.67 | $\Gamma_m$ = 3.8 mg/g |
| 1D | 0.0413 | 3.52 | 2.91 | |
| 1E | 0.0537 | 4.26 | 3.15 | K = 80.8 mL/mg |
| 1F | 0.0661 | 4.91 | 3.36 | |
| 1G | 0.0794 | 5.92 | 3.36 | |
| 1H | 0.0921 | 6.59 | 3.50 | |
| 1I | 0.1053 | 7.50 | 3.50 | |
| 1J | 0.1186 | 8.47 | 3.51 | |
| 2A | 0.0058 | 0.79 | 1.84 | |
| 2B | 0.0165 | 1.69 | 2.43 | |
| 2C | 0.0288 | 2.71 | 2.67 | $\Gamma_m$ = 3.8 mg/g |
| 2D | 0.0414 | 3.71 | 2.81 | |
| 2E | 0.0538 | 4.52 | 2.99 | K = 90.8 mL/mg |
| 2F | 0.0664 | 5.34 | 3.13 | |
| 2G | 0.0788 | 5.98 | 3.30 | |
| 2H | 0.0914 | 6.66 | 3.44 | |
| 2I | 0.1040 | 7.29 | 3.57 | |
| 2J | 0.1128 | 7.06 | 4.68 | |
| 3A | 0.0055 | 0.84 | 1.65 | |
| 3B | 0.0161 | 2.00 | 2.02 | |
| 3C | 0.0259 | 2.50 | 2.58 | $\Gamma_m$ = 4.3 mg/g |
| 3D | 0.0369 | 3.23 | 2.87 | |
| 3E | 0.0468 | 3.44 | 3.43 | K = 63.5 mL/mg |
| 3F | 0.0597 | 4.67 | 3.19 | |
| 3G | 0.0706 | 5.06 | 3.47 | |
| 3H | 0.0799 | 4.77 | 4.17 | |
| 3I | 0.0932 | 6.01 | 3.90 | |
| 3J | 0.1043 | 6.32 | 4.11 | |
| 4A | 0.0047 | 0.63 | 1.84 | |
| 4B | 0.0137 | 1.30 | 2.64 | |
| 4C | 0.0260 | 2.51 | 2.57 | $\Gamma_m$ = 4.3 mg/g |
| 4D | 0.0350 | 2.60 | 3.38 | |
| 4E | 0.0480 | 3.81 | 3.13 | K = 85.1 mL/mg |
| 4F | 0.0595 | 4.50 | 3.29 | |
| 4G | 0.0700 | 4.72 | 3.74 | |
| 4H | 0.0808 | 5.00 | 4.01 | |
| 4I | 0.0933 | 5.91 | 3.96 | |
| 4J | 0.1027 | 5.55 | 4.65 | |

SE#3F, 3827-59

| Sample | $C_e$, mg/mL | $C_e/C^*$ | Specific Sorption | |
|---|---|---|---|---|
| 1A | 0.0036 | 0.48 | 1.86 | |
| 1B | 0.0130 | 1.44 | 2.26 | |
| 1C | 0.0212 | 1.79 | 2.96 | $\Gamma_m$ = 3.5 mg/g |
| 1D | 0.0321 | 2.71 | 2.96 | |
| 1E | 0.0409 | 2.91 | 3.51 | K = 239.3 mL/mg |
| 1F | 0.0529 | 4.05 | 3.27 | |
| 1G | 0.0638 | 4.83 | 3.29 | |
| 1H | 0.0742 | 5.38 | 3.45 | |
| 1I | 0.0861 | 6.66 | 3.23 | |
| 1J | 0.0951 | 6.38 | 3.73 | |
| 2A | 0.0042 | 0.64 | 1.64 | |
| 2B | 0.0123 | 1.34 | 2.31 | |
| 2C | 0.0218 | 2.08 | 2.63 | $\Gamma_m$ = 3.4 mg/g |
| 2D | 0.0327 | 3.17 | 2.57 | |
| 2E | 0.0420 | 3.56 | 2.94 | K = 143.7 mL/mg |
| 2F | 0.0525 | 4.38 | 2.98 | |
| 2G | 0.0625 | 4.88 | 3.18 | |
| 2H | 0.0740 | 6.17 | 3.00 | |
| 2I | 0.0842 | 6.68 | 3.13 | |
| 2J | 0.0942 | 7.08 | 3.33 | |
| 3A | 0.0045 | 0.73 | 1.53 | |
| 3B | 0.0122 | 1.33 | 2.30 | |
| 3C | 0.0210 | 1.89 | 2.78 | $\Gamma_m$ = 3.3 mg/g |
| 3D | 0.0321 | 3.00 | 2.67 | |
| 3E | 0.0421 | 3.70 | 2.85 | K = 177.8 mL/mg |
| 3F | 0.0527 | 4.59 | 2.86 | |
| 3G | 0.0573 | 3.26 | 4.39 | |
| 3H | 0.0730 | 5.80 | 3.16 | |
| 3I | 0.0816 | 5.56 | 3.63 | |
| 3J | 0.0919 | 6.10 | 3.79 | |
| 4A | 0.0074 | 1.26 | 1.46 | |
| 4B | 0.0171 | 1.82 | 2.37 | |
| 4C | 0.0291 | 2.72 | 2.67 | $\Gamma_m$ = 3.3 mg/g |
| 4D | 0.0413 | 3.52 | 2.91 | |
| 4E | 0.0537 | 4.26 | 3.15 | K = 221.0 mL/mg |
| 4F | 0.0661 | 4.91 | 3.36 | |
| 4G | 0.0794 | 5.92 | 3.36 | |
| 4H | 0.0921 | 6.59 | 3.50 | |
| 4I | 0.1053 | 7.50 | 3.50 | |
| 4J | 0.1186 | 8.47 | 3.51 | |

SE#3G, 3827-63

| Sample | $C_e$, mg/mL | $C_e/C^*$ | Specific Sorption | |
|---|---|---|---|---|
| 1A | 0.0043 | 0.65 | 1.65 | |
| 1B | 0.0130 | 1.47 | 2.21 | |
| 1C | 0.0202 | 1.61 | 3.11 | $\Gamma_m$ = 4.0 mg/g |
| 1D | 0.0318 | 2.67 | 2.98 | |
| 1E | 0.0414 | 3.13 | 3.30 | K = 114.3 mL/mg |
| 1F | 0.0522 | 3.91 | 3.34 | |
| 1G | 0.0625 | 4.47 | 3.47 | |
| 1H | 0.0715 | 4.50 | 3.96 | |
| 1I | 0.0835 | 5.63 | 3.67 | |
| 1J | 0.0946 | 6.46 | 3.67 | |
| 2A | 0.0038 | 0.53 | 1.79 | |
| 2B | 0.0129 | 1.44 | 2.24 | |
| 2C | 0.0221 | 2.06 | 2.67 | $\Gamma_m$ = 4.1 mg/g |
| 2D | 0.0320 | 2.72 | 2.94 | |
| 2E | 0.0431 | 3.71 | 2.90 | K = 95.9 mL/mg |
| 2F | 0.0520 | 3.81 | 3.41 | |
| 2G | 0.0614 | 4.04 | 3.78 | |
| 2H | 0.0724 | 4.76 | 3.80 | |
| 2I | 0.0853 | 6.47 | 3.30 | |
| 2J | 0.0936 | 5.86 | 3.97 | |
| 3A | 0.0040 | 0.59 | 1.69 | |
| 3B | 0.0121 | 1.27 | 2.36 | |
| 3C | 0.0209 | 1.81 | 2.88 | $\Gamma_m$ = 4.3 mg/g |
| 3D | 0.0311 | 2.56 | 3.04 | |
| 3E | 0.0402 | 2.90 | 3.47 | K = 106.9 mL/mg |
| 3F | 0.0497 | 3.27 | 3.79 | |
| 3G | 0.0605 | 3.98 | 3.79 | |
| 3H | 0.0693 | 4.03 | 4.30 | |
| 3I | 0.0811 | 5.00 | 4.04 | |
| 3J | 0.0904 | 5.10 | 4.42 | |
| 4A | 0.0040 | 0.57 | 1.75 | |
| 4B | 0.0124 | 1.29 | 2.40 | |
| 4C | 0.0213 | 1.82 | 2.93 | $\Gamma_m$ = 4.5 mg/g |
| 4D | 0.0321 | 2.70 | 2.96 | |
| 4E | 0.0398 | 2.62 | 3.79 | K = 120.5 mL/mg |
| 4F | 0.0497 | 3.05 | 4.06 | |
| 4G | 0.0612 | 3.85 | 3.96 | |
| 4H | 0.0713 | 4.24 | 4.18 | |
| 4I | 0.0825 | 4.97 | 4.13 | |
| 4J | 0.0942 | 5.92 | 3.94 | |

SE#4, 3827-87

SE#4-1 MW ≈ 1.9 X $10^6$
SE#4-2 MW ≈ 0.7 X $10^6$
SE#4-3 MW ≈ 0.2 X $10^6$

| Sample | $C_e$, mg/mL | $C_e/C^*$ | Specific Sorption | |
|---|---|---|---|---|
| 1A | 0.0039 | 0.66 | 1.48 | |
| 1B | 0.0103 | 1.09 | 2.37 | |
| 1C | 0.0187 | 1.69 | 2.77 | $\Gamma_m$ = 4.4 mg/g |
| 1D | 0.0273 | 2.20 | 3.09 | |
| 1E | 0.0366 | 2.83 | 3.20 | K = 98.7 mL/mg |
| 1F | 0.0452 | 3.16 | 3.58 | |
| 1G | 0.0548 | 3.75 | 3.61 | |
| 1H | 0.0642 | 4.22 | 3.78 | |
| 1I | 0.0726 | 4.34 | 4.18 | |
| 1J | 0.0838 | 5.46 | 3.85 | |
| 2A | 0.0036 | 0.51 | 1.75 | |
| 2B | 0.0102 | 0.91 | 2.78 | |
| 2C | 0.0200 | 1.67 | 2.98 | $\Gamma_m$ = 4.5 mg/g |
| 2D | 0.0275 | 1.84 | 3.69 | |
| 2E | 0.0387 | 2.68 | 3.58 | K = 135.5 mL/mg |
| 2F | 0.0478 | 3.00 | 3.96 | |
| 2G | 0.0585 | 3.68 | 3.92 | |
| 2H | 0.0680 | 3.99 | 4.21 | |
| 2I | 0.0798 | 5.06 | 3.96 | |
| 2J | 0.0889 | 5.11 | 4.29 | |
| 3A | 0.0037 | 0.66 | 1.41 | |
| 3B | 0.0079 | 0.72 | 2.75 | |
| 3C | 0.0144 | 1.03 | 3.48 | $\Gamma_m$ = 5.2 mg/g |
| 3D | 0.0234 | 1.64 | 3.56 | |
| 3E | 0.0322 | 2.17 | 3.67 | K = 102.7 mL/mg |
| 3F | 0.0412 | 2.67 | 3.86 | |
| 3G | 0.0487 | 2.81 | 4.32 | |
| 3H | 0.0579 | 3.30 | 4.33 | |
| 3I | 0.0654 | 3.37 | 4.82 | |

# APPENDIX V

## ERROR ANALYSIS FOR SORPTION EXPERIMENT # 3

With the realization that the sample mean or average value (X) is only an estimate of the of the true value ($\mu$), the question arises as to how close X agrees with $\mu$. Statistically an interval around the mean can be defined within which $\mu$ would be expected to fall. The limits for which $\mu$ would have a certain probability of existence are referred to as confidence limits. They are calculated using Student's t distribution curve and the equation below:

$$\overline{X} \pm \frac{t \times s}{\sqrt{n}}$$

where t = value from distribution curve at a certain % probability level (95%)

s = standard deviation

n = number of measurements

The maximum specific sorption values from SE#3E-3G were examined using this type of analysis (see Table 18). Not all of the values fall within the 95% confidence interval so further analyses (using the standard error equation) were performed.

Table 18. Student's t test on SE#3E-3G maximum specific sorption data.

| Sample | $\Gamma_m$ | |
|---|---|---|
| SE#3E-1 | 3.9 | |
| 2 | 3.8 | $\bar{X} = 3.9$ |
| 3 | 4.3 | |
| 4 | 4.3 | $s = 0.4$ |
| SE#3F-1 | 3.5 | |
| 2 | 3.4 | $n = 12$ |
| 3 | 3.3 | |
| 4 | 3.3 | $t\ (95\%) = 2.201$ |
| SE#3F-1 | 4.0 | |
| 2 | 4.1 | $3.9 \pm 0.3$ |
| 3 | 4.3 | |
| 4 | 4.5 | $\bar{X} \pm \frac{t \times s}{\sqrt{n}}$ |

For the general case of $y = f(x_1, x_2, \ldots, x_m)$ where the dependent variable y is a function of the independent variables $x_1, x_2, \ldots, x_m$, the relative error in y due to the relative error in all the x's is given by [63]:

$$\left(\frac{\Delta y}{y}\right)^2 = \sum_{i=1}^{\infty} \left(\frac{\partial \ln f}{\partial \ln x_i}\right)^2 \left(\frac{\Delta x_i}{x_i}\right)^2$$

For confidence limits on $C_e/C^*$ let $f = C_e/C^*$. Then,

$$\left(\frac{\Delta f}{f_i}\right)^2 = \left(\frac{\partial \ln f}{\partial \ln C_e}\right)^2 \left(\frac{\Delta C_{e,i}}{C_{e,i}}\right)^2 + \left(\frac{\partial \ln f}{\partial \ln C_i}\right)^2 \left(\frac{\Delta C_{i,i}}{C_{i,i}}\right)^2$$

where $\Delta C_{e,i}$ = confidence limits on $C_e$

$\Delta C_{i,i}$ = confidence limits on the control samples

For $\partial \ln f / \partial \ln C_e$, let $\ln C_e = u$ and then $C_e = e^u$. After much manipulation:

$$\frac{\partial \ln f}{\partial \ln C_e} = \frac{C_i}{C_i - C_e}$$

For $\partial \ln f / \partial \ln C_i$, let $\ln C_i = u$ and then $C_i = e^u$. Again, after much manipulation:

$$\frac{\partial \ln f}{\partial \ln C_i} = \frac{C_i}{C_e - C_i}$$

This leads to a final equation of :

$$\left(\frac{\Delta f}{f_i}\right)^2 = \left(\frac{C_i}{C_i - C_e}\right)^2 \left(\frac{\Delta C_{e,i}}{C_{e,i}}\right)^2 + \left(\frac{C_i}{C_e - C_i}\right)^2 \left(\frac{\Delta C_{i,i}}{C_{i,i}}\right)^2$$

For confidence limits on $r_m$ as calculated by determining the error associated with the slope of the line obtained from the graph of $C_e/C^*$ vs. $C_e$, the following manipulations of the standard error equation is necessary. Since,

$$\Gamma_m = \frac{\left(m^{-1} \times m_s\right)}{m_f}$$

where $m$ = slope of the line

$m_s$ = volume in sorption tube = 40 mL

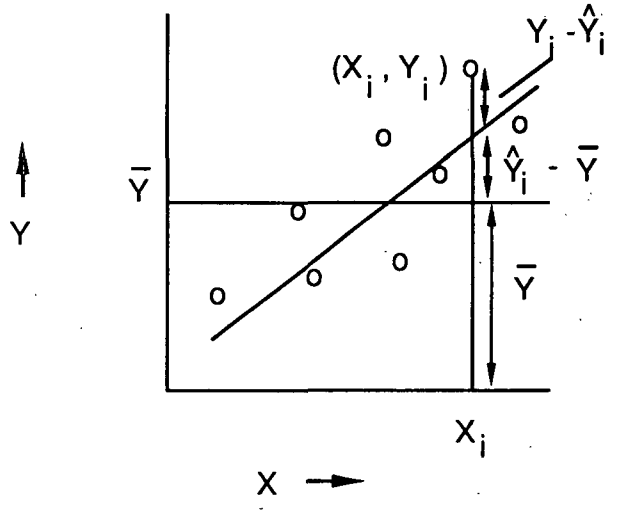
$m_f$ = weight of fiber = 0.16 g,

the confidence limits on $\Gamma_m$ ($\Delta \Gamma_m$) are calculated from:

$$\left(\frac{\Delta\Gamma_m}{\Gamma_m}\right)^2 = \left(\frac{\Delta m_s}{m_s}\right)^2 + \left(\frac{\Delta m_f}{m_f}\right)^2 + \left(\frac{\Delta m}{m}\right)^2$$

$\Delta m_s$ and $\Delta m_f$ are error due to weighing; $0.1 \times 10^{-3}$ g is the value given by the balance manufacturer's owner manual. [64] Figure 29 can be used to explain how $\Delta m$ is calculated. Then $\Delta \Gamma_m$ can be solved for.

Figure 29. Calculation of error associated with slope.




$$\Delta m = \pm \frac{t \times s}{\sqrt{\Sigma (X_i - \bar{X})^2}}$$

$$s = \sqrt{\frac{\Sigma (Y_i - \hat{Y}_i)^2}{n - 2}}$$

# APPENDIX VI

## SORPTION EXPERIMENT # 4; LANGMUIR PLOTS

Figure 30. Adsorption isotherm and Langmuir plot for SE#4-1.

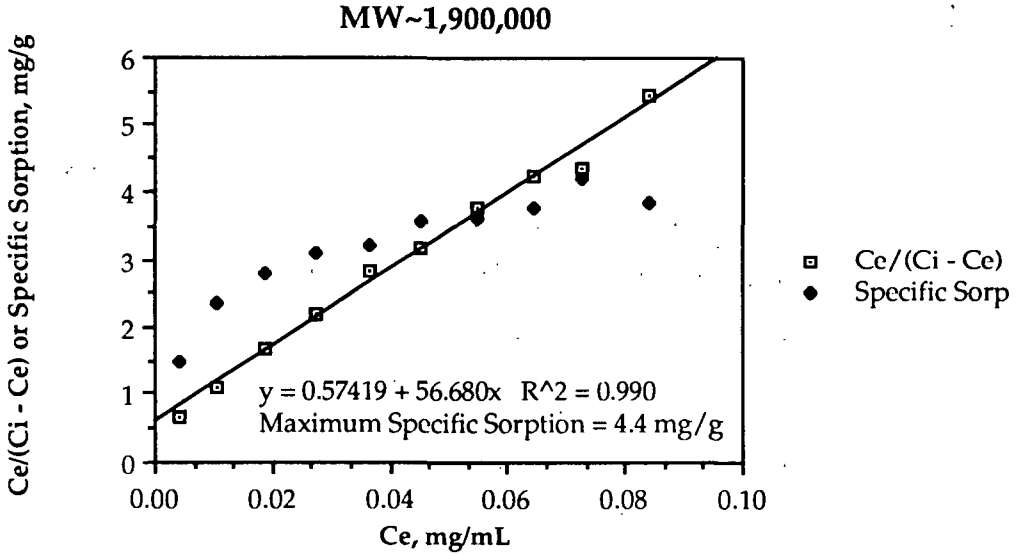



Figure 31. Adsorption isotherm and Langmuir plot for SE#4-2.

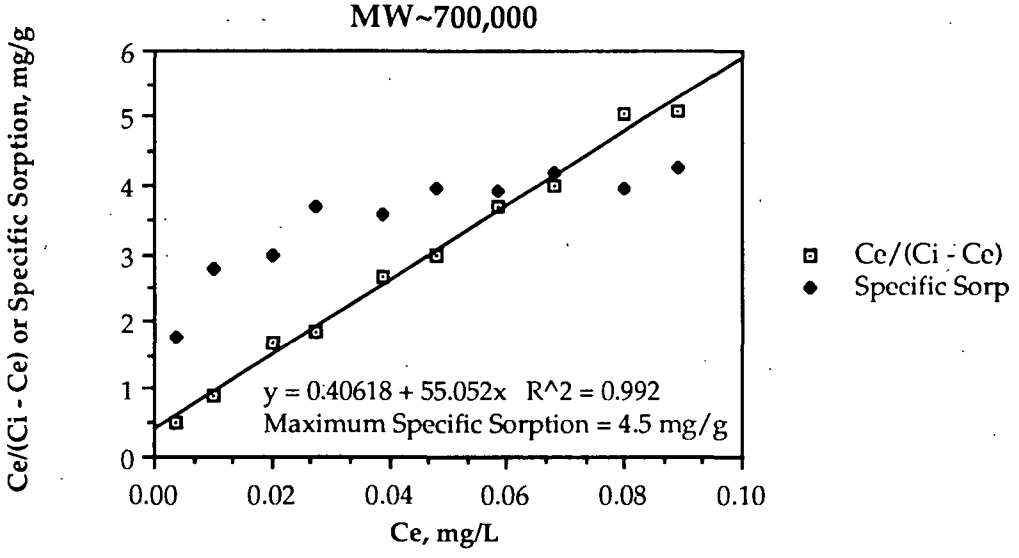


Figure 32. Adsorption isotherm and Langmuir plot for SE#4-3.

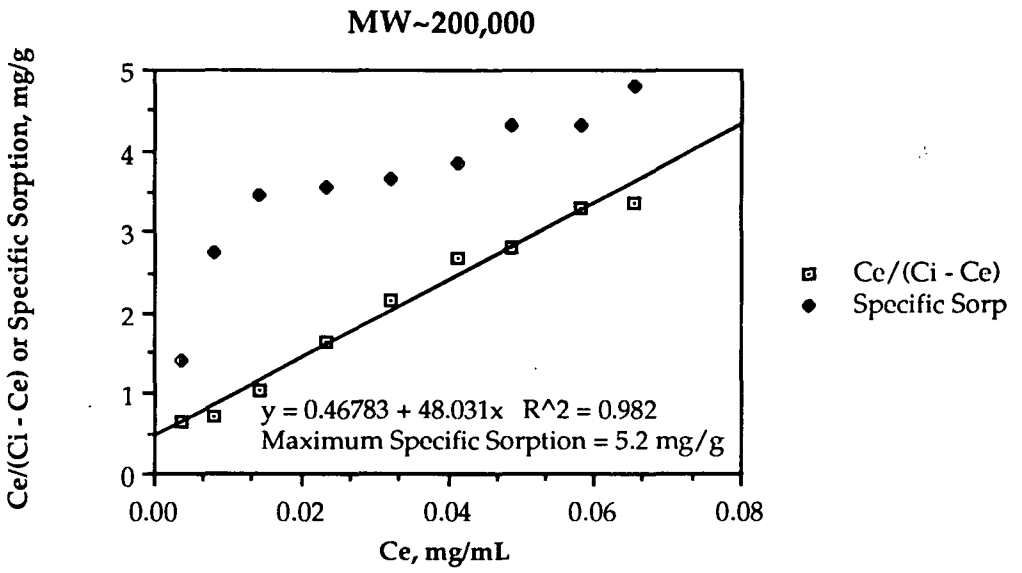
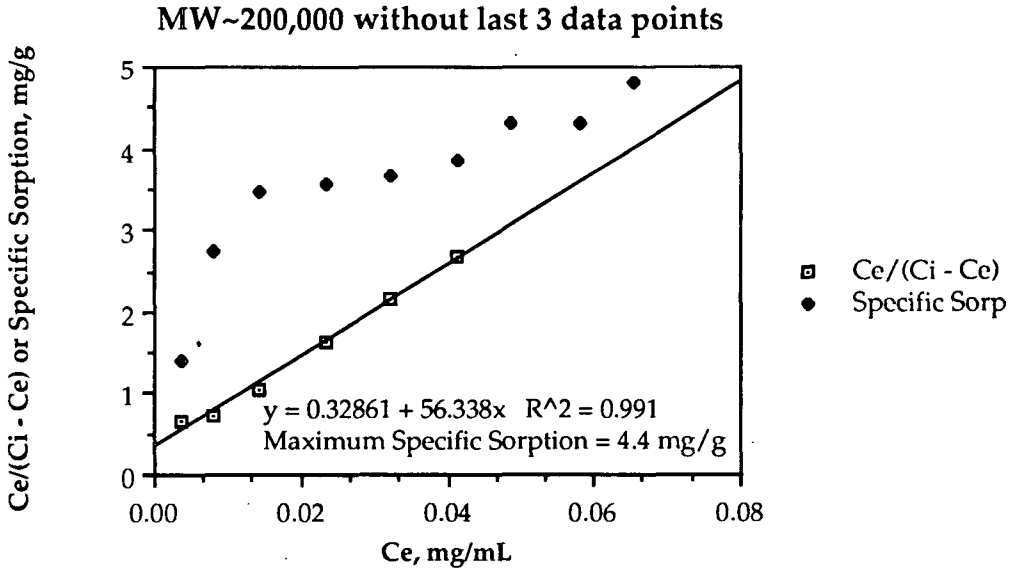



Figure 33. Adsorption isotherm and Langmuir plot for SE#4-3.



# APPENDIX VII

## EFFECT OF XYLOGLUCAN ON HANDSHEET PROPERTIES

Xyloglucan was applied to spruce semichemical mechanical pulp (SCMP; sodium sulfite/sodium hydroxide, 94.7% yield) by H. Grady of the I.P.C. Pulp Lab (Project #3566). Xyloglucan was added to the pulp slurry (5% overnight and 5% at 60°C for 1 hour) prior to sheet formation and as a 2% surface application. Handsheet properties were then measured by the I.P.C. Paper Evaluation Staff. Table 19 indicates that application of xyloglucan to this SCMP did not affect most standard handsheet properties. The tensile index did increase over the untreated, spruce SCMP, but still was much lower than that of an untreated, spruce kraft pulp.

Laffend [34] and Walker [36] also examined the effect of hemicellulose glucomannan and glucuronoarabinoxylan, respectively) sorption on standard handsheet properties. Both found considerable increases in strength properties over those of a cotton linters control pulp (see Tables 20 and 21). The deacetylated glucomannan gave a higher tear factor, breaking length, stretch, and tensile energy absorption than the acetylated polymer. Laffend [34] hypothesized that this indicated that the presence of acetyl groups had a deleterious effect on glucomannan's efficiency as a beater additive. The unreduced glucuronoarabinoxylan was found to give better strength properties than the 60% reduced polymer; the uronic acid carboxyls were believed to improve sheet strength properties because of their hydrophilic nature. [36]

Table 19. Handsheet properties of spruce SCMP with xyloglucan addition and spruce kraft.

| | Untreated SCMP | 5% XG in slurry overnight | 5% XG in slurry 60°C, 1 hr | 2%XG surface applied | Untreated Kraft |
|---|---|---|---|---|---|
| Density, g/cc (O.D.B.W.) | 0.128 | 0.143 | 0.131 | 0.141 | 0.378 |
| Tear Factor avg, gF/100g | 50.2 | 46.2 | 35.0 | 45.1 | 219.5 |
| 50% RH Tensile Index avg, Nm/g | 5.5 | 7.7 | 6.0 | 9.9 | 35.3 |
| Stretch avg, % | 0.8 | 0.7 | 0.5 | 0.8 | 1.75 |
| Et (stiffness) avg, lbs/inch | 802 | 512 | 512 | 630 | 1990 |
| Zero Span Index avg, Nm/g | 80.0 | 81.9 | 81.2 | 87.1 | 169.6 |

Table 20. Handsheet properties of cotton linter pulp with acetylated and deacetylated glucomannan addition.

| | Cotton Linter Control | Acetylated Glucomannan (≈ 6.7% retained) (MW ≈ 14,500) | Deacetylated Glucomannan (≈6.9% retained) (MW ≈ 15,900) |
|---|---|---|---|
| Tear Factor, g/100 g | 96.7 | 99.1 | 104.1 |
| Breaking Length, m | 1939.6 | 2746.9 | 3061.2 |
| Stretch, % | 2.29 | 4.42 | 4.72 |
| Tensile Energy Absorption, kg cm/cm$^2$ | 0.0242 | 0.0638 | 0.0760 |

Table 21. Handsheet properties of cotton linter pulp with reduced and unreduced gluronoarabinoxylan (GAX) addition.

| | Cotton Linter Control | Unreduced GAX (9.1 mg/g pulp) | Reduced GAX (16.1 mg/g pulp) |
|---|---|---|---|
| Tear, g | 63 | 71 | 71 |
| Instron Tensile, lb | 5.7 | 8.0 | 7.6 |
| Stretch, % | 2.39 | 2.43 | 2.17 |
| Tensile Energy Absorption, in. lb./sq. in. | 0.06 | 0.14 | 0.12 |